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Altered expression of G-protein mRNA in spontaneously hypertensive rats

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We have recently demonstrated that the decreased ability of hormones, forskolin and GTP to stimulate adenylate cyclase in heart and aorta from spontaneously hypertensive rats (SHR), as compared to their age-matched Wistar–Kyoto control rats (WKY), was associated with enhanced levels of Gi- and not with Gs-regulatory proteins. In the present studies we have investigated the expression of Gi-regulatory proteins at the mRNA level by Northern blotting. Total RNA of heart ventricle and aorta from WKY and SHR was probed with radiolabeled cDNA inserts encoding Giα-2 and Giα-3. The Giα-2 and Giα-3 probes detected a message of 2–3 and 3–5 kb, respectively, in both WKY and SHR, however, the message was significantly enhanced in SHR, as compared by WKY. On the other hand the cDNA probe encoding Gs_α detected a message of 1.8 kb in heart and aorta from both WKY and SHR, however, no difference in the levels of Gs_α mRNA was detected in SHR and WKY tissues. These results indicate that the mRNA levels of Giα-2 and Giα-3 and not of Gs are overexpressed in heart and aorta from SHR, which may be responsible for the increased levels of Gi as shown earlier by immunoblotting techniques. It may be suggested that the enhanced vascular tone and impaired cardiac contractility in hypertension may partly be the consequences of increased levels of Gi in heart and aorta.

G-protein; mRNA; Heart; Aorta; Hypertension

1. INTRODUCTION

Guanine nucleotide regulatory proteins (G-proteins) are a family of GTP-binding proteins that play an important role in the regulation of a variety of signal transduction systems. One of these is the adenylate cyclase/cAMP system, which is composed of three components: receptor, catalytic subunit, and stimulatory (Gs) and inhibitory (Gi) G-proteins which mediate the stimulatory and inhibitory responses of hormones on adenylate cyclase, respectively [1–3]. G-proteins exist as heterotrimers of α, β, γ subunits, and the specificity of G-proteins is attributed to the α-subunits. These Gsα and Giα subunits are known to be specifically ADP-ribosylated by cholera toxin and pertussis toxin, respectively [4]. Recently, molecular cloning has revealed four different forms of Gsα resulting from the differential splicing of one gene [5,6], and three distinct forms of Giα: Giα1, Giα2 and Giα3 encoded by three distinct genes [7–9].

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Abbreviations: Gs, stimulatory guanine nucleotide regulatory protein; Giα-1, inhibitory guanine nucleotide regulatory protein; Giα-1, Giα-2, Giα-3 isoforms of inhibitory guanine nucleotide regulatory protein encoded by these distinct genes; WKY, Wistar–Kyoto rats; SHR, spontaneously hypertensive rats, mRNA, messenger ribonucleic; rRNA, ribosomal ribonucleic acid.

The adenylate cyclase/cAMP system has been implicated in both the control of heart contractility [10,11] and the tone of vascular smooth muscles [12,13]. We have previously reported that forskolin, guanine nucleotides and some hormones were less responsive to stimulate adenylate cyclase in heart sarcolemma and aorta from spontaneously hypertensive rats (SHR) as compared to their Wistar–Kyoto controls (WKY) [14], suggesting that all three components of the adenylate cyclase system may be impaired in SHR. Further investigations on G-proteins, using bacterial toxin-catalyzed ADP-ribosylations and immunoblotting techniques, have demonstrated enhanced levels of Giα and not Gsα-protein in heart sarcolemma and aorta from SHR as compared to WKY rats, which were shown to be associated with an augmented inhibitory response of hormones on adenylate cyclase activity [15].

In order to investigate whether the enhanced levels of Giα, as demonstrated previously in SHR, are transcriptionally regulated, we undertook the present studies and analyzed the mRNA expression of Gi-proteins in heart and aorta from SHR and WKY rats, using cDNA probes for G-binding proteins.

2. MATERIALS AND METHODS

2.1. *Animals*

Male SHR and normotensive WKY rats of 12 weeks of age were purchased from Charles River Canada (Saint Constant, Que., Canada). Their blood pressure, measured by the tail cuff method without anesthesia, was 98.5 ± 3.3 and 146.5 ± 5.9 mmHg for WKY and SHR

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groups, respectively. Body weights were 172.5 ± 1 9 and 196.4 ± 1 9 g, respectively.

2.2. *Materials*

Plasmids containing rat cDNAs encoding Giα2, Giα3 and Gsα were kindly obtained from Dr Randall Reed from the Johns Hopkins University, and Dr. Hiroshi Itoh from the University of Tokyo. The 32-mer oligonucleotide which recognizes a highly conserved region in the 28 S ribosomal RNA was kindly donated by Dr. Yoshihiro Ishikawa from Lederle Laboratory of New York. Chemicals necessary for total RNA extraction and Northern blot analysis were obtained from Sigma Chemical Co. (St. Louis, MO, USA), except guanidinium thiocyanate which was from Research Organics Inc. (Cleveland, OH, USA), and glyoxal from BDH Ltd. (St. Laurent, Que., Canada). Enzymes used for radiolabeling of cDNA probes were obtained from BRL (Burlington, Ont., Canada) and other chemicals were from Pharmacia Ltd. (Baie d'Urfée, Que., Canada). Nylon filter (Hybond-N), [α-^{32}P]dCTP (3,000 Ci/mmol) and [γ-^{32}P]ATP (3,000 Ci/mmol) were purchased from Amersham Corp. (Oakville, Ont., Canada).

2 3. *Total RNA extraction*

Total RNA was isolated by the guanidinium thiocyanate–phenol–chloroform method described by Chomczynski et al. [16]. Briefly, frozen aorta and heart ventricles were homogenized in a denaturating solution (solution D) containing 4 M guanidinium thiocyanate, 25 mM sodium citrate, pH 7 0, 0 5% sarkosyl and 0 1 M 2-mercaptoethanol The homogenates were extracted once with 1 vol. of phenol and 0.2 vol. of cloroform–isoamylalcohol (49·1) in the presence of 0 2 M sodium acetate, pH 4.0, and once with 1 vol of chloroform–isoamylalcohol (49:1) Total RNA was then precipitated with isopropanol. Following a second precipitation in solution D and isopropanol (v/v), total RNA was washed in 70% ethanol and resuspended in water.

2.4 *Radiolabeling of the probes*

cDNA inserts encoding for Giα2, Giα3 and Gsα were radiolabeled with [α-^{32}P]dCTP by random priming essentially as described by Feinberg et al. [17]. Specific activities of the labeled probes ranged from 1 to 3 · 10^8 cpm/μg of DNA. The 32-mer oligonucleotide recognizing the 28 S rRNA was end-labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase as described by Sambrook et al. [18].

2 5. *Northern analysis*

DMSO/glyoxal-treated total RNA was resolved on 1% agarose gels and transferred to nylon membrane as described by Sambrook et al [19]. Filters, after prehybridization at 65°C for 6 h in hybridization solution (600 mM NaCl, 8 mM EDTA, 120 mM Tris, pH 7 4, 0 1% sodium pyrophosphate, 0.2% SDS and 500 U/ml heparin), 0.1% sodium pyrophosphate, 0.2% SDS, heparin 500 U/ml, were hybridized overnight in hybridization solution containing dextran sulphate (10% w/v) and the cDNA probe at 1–3 · 10^6 cpm/ml as described by Singh et al. [20] Filters were then rinsed at 65°C for 2 × 30 min in 300 mM NaCl, 4 mM EDTA, 60 mM Tris, pH 7.4, and 0.2% SDS, and 1 × 30 min in 150 mM NaCl, 2 mM EDTA, 30 mM Tris, pH 7.4, and 0 1% SDS. Autoradiography was performed with X-ray films at −70°C. In order to assess the possibility of any variations in the amounts of total RNA in individual samples applied to the gel, each filter was hybridized with the ^{32}P end-labeled oligonucleotide, which recognizes a highly conserved region of 28 S ribosomal RNA. The blots which had been probed with the G-protein cDNA were de-hybridized by washing for 1 h at 65°C in 50% formamide, 300 mM NaCl, 4 mM EDTA and 60 mM Tris, pH 7.4, and re-hybridized overnight at room temperature with the oligonucleotide. Quantitative analysis of the hybridization of probes bound was performed by densitometric scanning of the autoradiographs employing the enhanced laser densitometer, LKB Ultroscan XL and quantified using the gel scan XL evaluation software (version 2.1) from Pharmacia (Que., Canada).

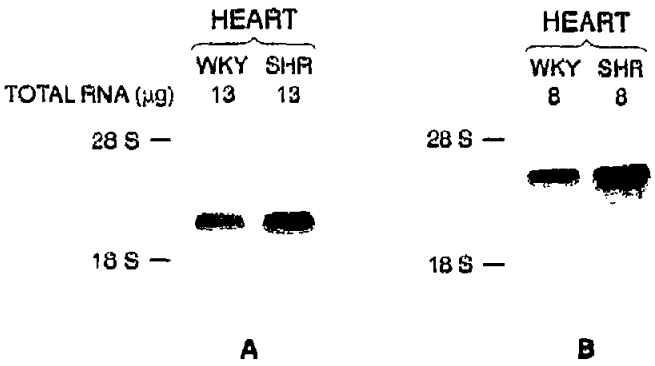

Fig 1. mRNA expression of Giα2 and Giα3 in heart ventricles from SHR and WKY rats. Total RNA of heart ventricles isolated from WKY (n = 3) and age-matched SHR (n = 3) were subjected to 1% agarose gel electrophoresis and transferred to nylon membrane. The blots were then probed with a full-length radiolabeled Giα2 cDNA probe (A) or with a full-length radiolabeled Giα-3 cDNA probe (B) as described in section 2

3. RESULTS AND DISCUSSION

We have recently demonstrated the over expression of Giα in heart sarcolemma and aorta from SHR by PT-catalyzed ADP-ribosylation and immunoblotting techniques using specific antibodies against different isoforms of Giα [15]. In the present studies we have investigated whether the enhanced levels of Giα protein observed previously in SHR were due to an increased synthesis of Giα proteins. The abundance of the different mRNAs and rRNA was determined by Northern rather than dot blotting to ensure that we quantified only the probes bound to the specific transcript under all conditions.

Total RNA of heart ventricle was probed with radiolabelled cDNA inserts encoding for Giα2 and Giα3 which are known to be expressed in rat heart [8,21]. The mRNA expression of Giα2 and Giα3 are shown in Fig. 1. The Giα2 probe (Fig. 1A) detected a message of 2.3 kb in both WKY and SHR, however, it was significantly more expressed in SHR as compared to WKY. The Giα2 mRNA levels were found to be increased by about 60% in heart from SHR as compared to WKY (Fig. 2). Previous studies at the protein level have shown an about 50% increase in Giα2 levels [15], suggesting that the enhanced protein levels of Giα2 may be mainly attributed to an overexpression of Giα2 gene in heart from SHR. Similarly, the Giα3 probe (Fig. 1B) hybridized to a message of 3.5 kb and as observed for Giα2, the Giα3 mRNA levels were significantly higher in SHR as compared to WKY. Quantification of Giα3 mRNA in heart showed an about 120% increase in SHR as compared to WKY (Fig. 2) which does not match with an increase in Giα3 at protein level (~30%) as reported previously [15]. This observation is similar to the results reported in adipocytes from streptozotocin-induced rats, where a large increase in Giα3 mRNA levels was not matched by increases at the protein level [22]. Sim-

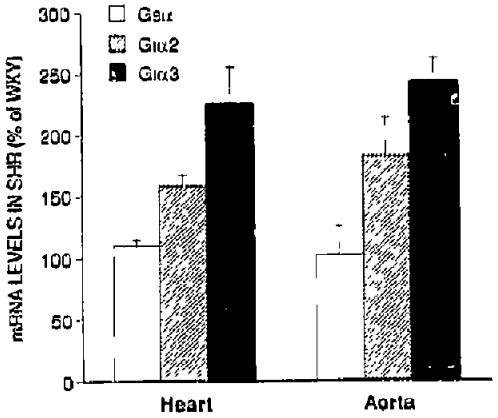

Fig. 2 Summary of the quantification of Northern blot by densitometric scanning. Northern blot of total RNA from heart and aorta of SHR and WKY rats were probed with radiolabeled cDNA probes as described in Section 2. Values obtained from the densitometric scans of the resulting autoradiographs were normalized relative to the amount of transcripts detected in the tissues from WKY rats which was arbitrarily assigned the value of 100%. The values for RNA from SHR tissues are given as a percentage of WKY values Results represent the mean ± S.E.M. of 4 or 5 separate experiments conducted with different preparations of total RNA.

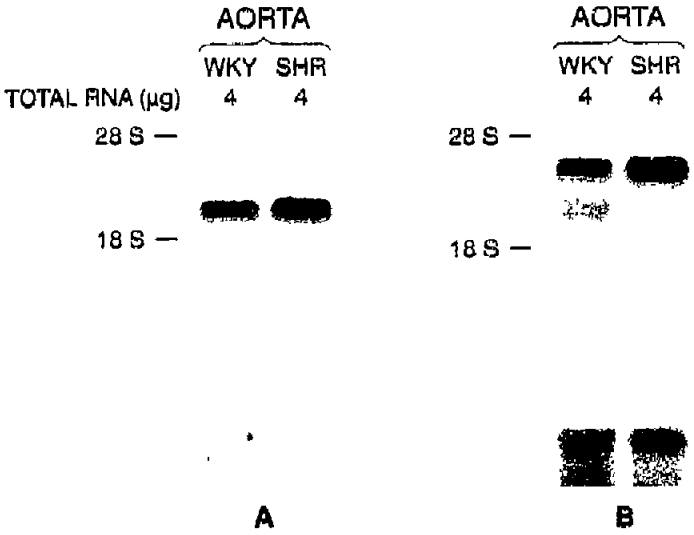

Fig. 3. mRNA expression of Giα2 and Giα3 in aorta from SHR and WKY rats. Total RNA of aortas isolated from WKY (n = 6) and age-matched SHR (n = 6) were analyzed by Northern blotting as described in Fig. 1 The blots were hybridized with the Giα2 cDNA probe (A) or with the Giα3 cDNA probe (B) (upper panels) and re-hybridized with an oligonucleotide recognizing the 28 S rRNA (lower panels).

ilarly Watford and Tatro [23] have also shown an increased number of transcripts for intestinal phosphoenolpyruvate carboxykinase in diabetes without any comparable increase at the protein level.

Since the aorta from SHR has been shown to exhibit similar alteration in the levels of Giα and adenylate cyclase activity as observed in heart [15], the mRNA expression of Giα2 and Giα3 was also examined in aortas from WKY and SHR and the results are shown in Fig. 3. The Giα2 and Giα3 probes detected 2.3 kb and 3.5 kb messages, respectively, in aorta from SHR and WKY, however, the Giα2 (Fig. 3A) and Giα3 (Fig. 3B) mRNA levels were also significantly higher in SHR as compared to WKY. Densitometric analysis of the blots showed about 80% increase in Giα2 mRNA levels and about 140% increase in Giα3 mRNA levels in aorta from SHR (Fig. 2) A similar increase in Giα2 levels as determined by immunoblotting has been shown previously [15], however, as observed in heart, the expression of Giα3 mRNA in aorta is also much higher than the increase at the protein level [15]. The alterations in Giα2 and Giα3 mRNA levels in heart and aorta from SHR may not be attributed to the variation in the amounts of total RNA in individual samples applied to the gels, due to the fact that the hybridization with an oligonucleotide that recognizes a highly conserved region of the 28 S rRNA showed a similar amount of 28 S rRNA loaded from WKY and SHR on to the gels. These data suggest that the enhanced expression of Giα proteins in heart and aorta from SHR as reported earlier [15] may be regulated at the transcriptional level. The regulation of Giα protein at transcriptional levels has also been reported in other conditions, such as diabetes [22] and human heart failure [24].

Since Giα-2 and not Giα-3 is involved in the coupling of inhibitory hormone receptors to adenylate cyclase [25–27], the increased expression of Giα-2 in heart and aorta may partly explain the augmented responsiveness of adenylate cyclase to inhibitory hormones in SHR [15]. On the other hand, Feldman et al. [24] were not able to detect Giα-2 mRNA in hearts from human heart failure but, consistent with our studies, have reported an increased level of Giα-3 mRNA and enhanced inhibition of adenylate cyclase in these hearts. A role for Giα-3 in gating K^+ channels has been established [28] and it would be interesting to examine if the increased levels of Giα-3 mRNA in SHR are also reflected in its functions.

We have also examined the Gsα protein expression at the transcriptional level in heart and aorta from WKY and SHR, and the mRNA levels of Gsα were determined using a cDNA probe coding for Gsα. As shown in Fig. 4, the Gsα cDNA probe detected a message of 1.8 kb in heart (A) and aorta (B) from both SHR and WKY, and the amount of Gsα mRNA was not significantly different in SHR and WKY rats (Fig. 2). These results are consistent with our previous data indicating that the levels of Gsα were not altered in SHR tissues as compared to WKY. However, Feldman et al. [24] despite reporting no change in the levels of Gsα protein in human heart failure, have shown an alteration in Gsα mRNA levels in this model. On the other hand, Chen et al. [29] have recently established a correlation between the decreased stimulation of adenylate

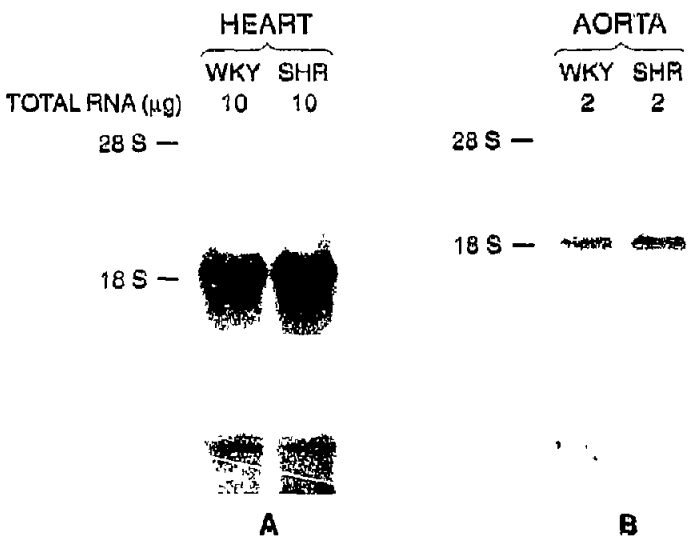

Fig. 4. mRNA expression of Gsα in SHR and WKY heart ventricles and aortas. As described in Fig. 1, total RNA from heart ventricles from WKY and SHR were probed with a full-length radiolabeled Gsα cDNA probe (upper panel) and further re-hybridized with an oligonucleotide recognizing the 28 S rRNA (lower panel). Total RNAs from aorta from SHR and WKY aortas (B) were treated as indicated in Fig 1. Blots were probed with Gsα cDNA probe (upper panel) and with the oligonucleotide recognizing the 28 S rRNA (lower panel).

cyclase and the decreased level of Gsα at the protein and mRNA levels in dog heart failure. Taken together, it is suggested that the expression of Gsα protein may be regulated by multiple mechanisms.

In conclusion, the present studies and several other previous reports support the idea that alteration in G-protein levels may result in altered signal transduction and thus have several implications for the development of different pathophysiological conditions, such as cardiovascular diseases [30,31], diabetes [32], and hypothyroidism [33]. To our knowledge, the present study is the first report demonstrating alterations in G-proteins at mRNA levels in hypertension. Whether the alteration in G-protein results in or partly induces the elevation of blood pressure in SHR has still to be explored. Chen et al. [29], however, have recently suggested that alteration in signal transduction may be involved in the genetic changes required for a cardiocyte to hypertrophy. In addition, different contractile and structural genes principally expressed in neonatal and fetal periods are known to be reactivated with the establishment of hypertrophy [34]. It is also known that hypertension is associated with alterations in the plasma levels of several hormones, such as catecholamines and atrial natriuretic factor [35,36], which may regulate the gene expression of G-proteins. The regulation of G-protein expression by thyroid [37] and steroid [38,39] hormones has been demonstrated. Sims et al. [40] have recently reported an increased expression of Gi at the protein level by angiotensin infusion. In the light of these observations, it would be exciting to explore whether the expression of G-proteins could be regulated by hormones and other factors which play an important role in the regulation of blood pressure.

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