Attenuation of changes in G_i -proteins and adenylyl cyclase in heart failure by an ACE inhibitor, imidapril

R. Sethi^a, Q. Shao^a, N. Takeda^b, N.S. Dhalla^{a*}

*^aInstitute of Cardiovascular Sciences, St. Boniface General Hospital Research Centre
& Department of Physiology, Faculty of Medicine, University of Manitoba,
Winnipeg, Canada,
and ^bAoto Hospital, Department of Internal Medicine, Jikei University,
Tokyo, Japan*

Received: September 10, 2003; Accepted: September 18, 2003

Abstract

Cardiac dysfunction in animals with congestive heart failure due to myocardial infarction (MI) is known to be associated with a wide variety of defects in receptor and post-receptor mechanisms. Since the heart function have been shown to be improved by treatment with different angiotensin converting enzyme (ACE) inhibitors, we examined the effects of imidapril, an ACE inhibitor, on changes in post-receptor mechanisms involving adenylyl cyclase (AC) and G proteins in the failing heart. Heart failure in rats was induced by occluding the coronary artery and 3 weeks later the animals were treated daily with 1 mg/kg (orally) imidapril for 5 weeks. The animals were assessed for their left ventricular function and crude membranes were isolated from the viable left ventricle and examined for AC activities as well as G-protein activities and expression. Animals with heart failure exhibited depressions in ventricular function and AC activities in the absence or presence of forskolin, NaF and Gpp(NH)p. The AC activity in the presence of pertussis toxin was increased whereas that in the presence of cholera toxin was decreased in the failing heart. Protein contents and mRNA levels for G_i -proteins were increased whereas those for G_s -proteins were unaltered in the infarcted heart. All these changes due to MI were prevented by imidapril treatment. The results indicate that the depressed cardiac function in the failing heart may partly be due to the direct effects of changes in AC and G_i proteins.

Keywords: myocardial infarction • congestive heart failure • angiotensin converting enzyme • G proteins, adenylyl cyclase • β -receptor density

* Correspondence to: Dr. Naranjan S. DHALLA,
Institute of Cardiovascular Sciences
St. Boniface General Hospital Research Centre
R3021 - 351 Tache Avenue

Winnipeg, MB R2H 2A6 Canada
Tel.: (204) 235-3417; Fax: (204) 233-6723
E-mail: nsdhalla@sbrc.ca

Introduction

It is now well established that catecholamines by acting on β -adrenoceptors (β -ARs) increase the formation of cyclic AMP through the stimulation of adenylyl cyclase (AC) and thus produce the positive inotropic effect on the heart. It is also known that β -ARs are coupled with AC through guanine nucleotide binding proteins (G-proteins), and these represent three essential components of the signal transduction pathway in the myocardium [1-3]. Defects in the β -AR signal transduction system in heart failure have been reported to be due to alterations in both stimulatory (G_s) and inhibitory (G_i) G-proteins [4,5]. Several studies have shown that the quantities of G_s - and G_i -proteins are altered by chronic agonist-induced activation of either stimulatory or inhibitory receptors coupled to AC [6-10]. In fact, the depression in the catecholamine-induced stimulation of the AC activity was found to be due to either a decrease of G_s -protein [8] or an increase of G_i -protein contents [6]. It is thus obvious that G-proteins which stimulate or inhibit the adenylyl cyclase activity may play a crucial role in regulating the heart function. Accordingly any change in the content and activity of the G_s -protein or G_i -protein under pathological conditions can be seen to modify the signal transduction mechanism in the heart. In this regard, alterations in G-proteins in terms of activities, protein contents and mRNA levels in different types of heart failure in human and various animal species have also been reported [11-16]. Since renin-angiotensin system (RAS) is known to play an important role in maintaining cardiovascular homeostasis and is considered to be involved in the pathophysiology of myocardial infarction (MI) [17], extensive efforts have been made to improve heart function in the infarcted animals with various RAS inhibitors. Treatment of rats with different angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor (AT1R) antagonists has been shown to prevent ventricular remodelling, improve heart function, change hormone profile and reduce mortality in the infarcted animals [18-22]. Although the cellular and molecular mechanisms by which these drugs improve cardiac function in heart failure have not yet been clearly defined, Shao et al [23] have demonstrated the effect of ACE inhibition on sar-

coplasmic reticular gene expression whereas Wang et al [24] demonstrated that the beneficial effects on the failing heart are associated with prevention of changes in myosin heavy chain isozyme protein contents and gene expression. Furthermore, recently the role of RAS in the phospholipase C-mediated signalling in congestive heart failure (CHF) has also been suggested [25]. Although defects in the β -ARs, G-proteins and AC [1-3,26,27] are known to occur in the failing heart, very little information regarding the effects of treatment with ACE inhibitors on this signal transduction mechanism is available in the literature.

In order to provide evidence whether or not post-receptor sites serve as a target for the action of ACE inhibitors, it was considered important to examine the effect of RAS inhibition on changes in AC activity and G-proteins in CHF. Because the coronary artery ligation model in rats is considered to exhibit several pathophysiologic characteristics that are similar to the clinical syndrome of human heart failure due to MI [21], the present study employed myocardial infarcted rats to examine the effect of an ACE inhibitor, imidapril, on post receptor mechanisms in the failing heart. The selection of imidapril for this study was mainly due to the fact that this long-acting agent has been reported to reduce mortality in a small coronary artery diseased animals to a greater extent than other ACE inhibitors and has been used previously in our laboratory [24,25,28]. Our results demonstrate that ACE inhibition was able to improve the cardiac function and attenuate the changes in AC activity and G-proteins in CHF due to MI.

Methods

All studies conform to the guidelines of the Animal Ethics Committee of the University of Manitoba.

Experimental model

MI was induced in male Sprague-Dawley rats (200-250 g) by occlusion of the coronary artery as described earlier [29,30]. The animals were anesthetized with isoflurane (5% mixed with O_2 , 2l/min),

the coronary artery was ligated approximately 2 mm from its origin with a suture of 6-0 silk, and the heart was repositioned in the chest. Rats were maintained on a positive pressure ventilation system delivering a mixture of 95% oxygen - 5% carbon dioxide mixed with isoflurane throughout the course of the operation. The mortality of animals with coronary ligation was approximately 40% within 48 hr. Sham operated animals were operated similarly except that the suture around the coronary artery was not tied. Rats were randomly divided into four groups: Sham (Control), Infarcted (Untreated), Sham (Treated) and Infarcted (Treated). Three weeks after occluding the coronary artery, the animals were treated with 1 mg/kg/day imidapril for 5 weeks unless specified otherwise; the sham control group and untreated infarcted group received saline injections and sham treated group received 1 mg/kg/day imidapril. Imidapril was given orally and its dose is same as that used in our previous studies [24].

Hemodynamic studies

The animals were anesthetised with an intraperitoneal injection of a mixture of ketamine (60 mg/kg) and xylazine (10 mg/kg), the right carotid artery was exposed and cannulated with a microtip pressure transducer (Model SPR-249, Millar Instruments, Houston, TX). The left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP), heart rate (HR) and rate of pressure development (+dP/dt) and rate of pressure decay (-dP/dt) were measured using the computer program (AcqKnowledge for Windows 3.0 Harvard, Montreal, Canada) [30]. The values for left ventricular developed pressure (LVDP) were calculated by subtracting the values for LVEDP from those for LVSP. Isoproterenol (1 µg/kg) was injected through the jugular vein after the basal hemodynamic parameters were measured.

Crude membranes preparation

The viable left ventricle (including septum) after the removal of scar was processed for the preparation of crude membranes according to the method described by Sethi et al [29]. Briefly, the tissue was

washed, minced and then homogenized in 50 mM Tris-HCl, pH 7.4 (15 ml/g tissue) with a PT-3000 polytron (2 x 20 sec, setting for 5 min) and the homogenate was centrifuged at 1000 g for 10 min and the pellet discarded. The supernatant was centrifuged at 48,000 g for 25 min, the resulting pellet was resuspended and centrifuged again twice in the same buffer at the same speed. The final pellet was resuspended in 50 mM Tris-HCl, pH 7.4, frozen rapidly in liquid nitrogen, and stored at -70°C.

Measurement of AC activity

The AC activity in the absence (basal) and presence of different stimulants such as forskolin, NaF and Gpp(NH)p was determined by measuring [³²P]-cAMP formation from [α-³²P]ATP as described previously [29]. The incubation in assay medium contained 50 mM glycylglycine (pH 7.5), 0.5 mM mgATP, [α-³²P] (1-1.5 x 10⁶ cpm), 5 mM MgCl₂ (in excess of the ATP concentration), 100 mM NaCl, 0.5 mM cAMP, 0.1 mM EGTA and an ATP regenerating system consisting of 2 mM creatine phosphate, 0.1 mg creatine kinase/ml and 0.1 mg myokinase/ml in a final volume of 200 µl. Incubations were initiated by the addition of (30 - 70 µg) membrane to the reaction mixture that has been equilibrated for 3 min at 37°C. The reactions, carried out in triplicate for 10 min at 37°C, were terminated by addition of 0.6 ml of 120 mM zinc acetate. Under the assay conditions employed in this study, AC activity in the presence or absence of different stimulants was linear with respect to protein concentration and the time of incubation. For studying the effects of pertussis and cholera toxins on the adenylyl cyclase activity, the membrane preparations were treated with or without toxin for 60 min at 30°C in the reaction mixture containing 10 mM NAD. The membrane preparation was washed twice with 50 mM Tris HCl buffer (pH 7.4) and suspended finally in the same buffer used for estimating the adenylyl cyclase activity. Since the ADP ribosylation turns on or off G-protein function, the measurement of adenylyl cyclase activity in the presence or absence of ADP ribosylation with either pertussis toxin or cholera toxin eliminates the role of numerous other proteins which may regulate the function of this system *in vivo*.

Immunoblotting for G_{ia} and G_{sa} contents

Immunoblotting was performed as described by Sethi et al. [30]. The blots were incubated with specific antisera [AS/7 specific for G_{ix}] (1;3000) overnight at 4°C and then washed several times for a 15 min duration with 10 mM tris-HCl, 150 mM NaCl, 0.05% Tween 20 (TBST) to remove any non-specifically bound antibodies. Antigen-antibody complexes were then visualized by incubating the membrane with 0.1 µCi/ml of 125I-protein A in TBST wash buffer for 1 hr at room temperature. Autoradiograms were developed and scanned with a densitometer to determine the relative intensity of the 42 kD band for G_{ia}-proteins and 45 kD as well as 52 kD bands for G_{sa}-proteins. Although other isoforms of G_i- and G_s-proteins are also expressed in the heart, no effort was made to determine their contents for the purpose of this study.

RNA isolation and Northern blot analysis

Total RNA from frozen ventricular tissue samples was prepared according to the protocol of Chomczynski and Sacchi [31], using the guanidinium thiocyanate-phenol-chloroform extraction procedure. Scanning densitometry was performed on autoradiograms to estimate the abundance of each particular mRNA species in control and experimen-

tal groups. Relative glyceraldehyde - 3 - phosphate dehydrogenase (GAPDH) mRNA and 18S RNA abundance were used as internal standards to normalize the density values for target mRNA levels.

Statistical analysis

Results are presented as mean ± S.E. The statistical differences between the mean values for the control and experimental groups were evaluated by the unpaired Student's t-test. When appropriate, Duncan's multiple range test was used to determine the differences between the multigroup means. A "P" value < 0.05 was considered to reflect a significant difference between groups.

Results

General characteristics of sham control, untreated and imidapril treated infarcted animals are given in Table 1. Cardiac hypertrophy in the untreated infarcted animals was evident by an increase in the mass of whole heart wt. Lung congestion as reflected by lung wet wt/dry wt ratio was also seen in the untreated infarcted animals indicating clinical signs of congestive heart failure in the 8 week infarcted animals. Treating the 3 week infarcted animals with imidapril (1 mg/kg for 5 weeks, oral/daily) attenu-

Table 1 General characteristics and hemodynamic changes in infarcted animals treated with or without imidapril.

	Sham	MI	Sham + IMP	IMP + MI
Body wt (g)	481 ± 8.7	488 ± 7.5	478 ± 10.2	472 ± 8.5
Heart wt (mg)	1120 ± 15.6	1532 ± 34.1*	1136 ± 18.5	1272 ± 16.9†
Scar wt (mg)	-	308 ± 14.9	-	307 ± 16.2
Lung wet/dry wt ratio	3.91 ± 0.15	556 ± 0.24*	4.10 ± 0.12	4.71 ± 0.18†
LVEDP (mm Hg)	3.2 ± 0.2	15.7 ± 1.7*	3.9 ± 0.3	7.6 ± 0.6†
LVDP (mm Hg)	122 ± 4.35	98 ± 3.5	125 ± 4.0	116 ± 4.5†
+dP/dt (mm Hg/sec)	7480 ± 301	4420 ± 271*	7256 ± 290	5816 ± 248†
-dP/dt (mm Hg/sec)	7842 ± 318	4764 ± 292*	758 ± 304	6204 ± 226+

Values are mean ± SE of 8 animals in each group. MI, infarcted animals; IMP, imidapril-treated group; LVEDP, left ventricular end diastolic pressure; LVDP, left ventricular developed pressure; +dP/dt, rate of pressure development; -dP/dt, rate of pressure decay. * - significantly (P<0.05) different from sham; † - significantly (P<0.05) different from MI.

Table 2 Effect of imidapril treatment for 5 weeks on the left ventricular adenylyl cyclase activity in 3 weeks infarcted rats.

	Adenylyl cyclase activity (pmol cAMP/mg protein/min)			
	Sham	MI	Sham + IMP	IMP + MI
Basal	33 ± 1.8	21 ± 1.2*	32 ± 2.1	39 ± 2.3 [†]
Forskolin (100 µM)	543 ± 28	404 ± 20*	568 ± 31	575 ± 32 [†]
NaF (5 mm)	308 ± 18	200 ± 14*	293 ± 21	283 ± 14 [†]
Gpp(NH)p (30 µM)	197 ± 13	129 ± 16*	239 ± 19	202 ± 17 [†]

Values are mean ± SE of 6 animals in each group. MI, infarcted animals; IMP, imidapril treated group. * - significantly (P<0.05) different from respective sham; [†] - significantly (P<0.05) different from respective infarcted group.

ated changes in these parameters. However, the scar wt in the untreated group was not different from that in the treated animals with MI. Data in Table 1 also show that LVEDP was increased whereas both +dp/dt and -dp/dt and LVDP were depressed in the untreated infarcted animals; these changes in heart function were attenuated in the infarcted animals upon treatment with imidapril. The administration of imidapril for 5 weeks to sham control animals did not alter the general characteristics or ventricular performance of these animals significantly (Table 1).

Because the AC activity is altered in the infarcted animals [27-30], we examined the effectiveness of imidapril treatment on changes in AC activity in control and infarcted animals. Data in Table 2 show that the basal AC and forskolin-stimulated AC activity of the crude membranes from viable left ventricle from the infarcted animals was decreased;

this change was attenuated by imidapril treatment for 5 weeks starting after 3 weeks of MI. To determine whether the observed alterations in the infarcted and imidapril treated hearts involve G-proteins, the enzyme activities were studied in the absence or presence of various concentrations of Gpp(NH)p and NaF. It should be noted that Gpp(NH)p, a nonhydrolyzable analog of GTP, increases the adenylyl cyclase activity by stimulating G_s-proteins directly, whereas NaF affects the AC by activating G_s-proteins [32]. The data in Table 2 also indicate that the enzyme activity in the presence of Gpp(NH)p, forskolin and NaF was depressed in crude membrane preparations from the infarcted hearts when compared to controls; membranes from the infarcted animals treated with imidapril however showed higher enzyme activity under similar conditions. In order to assess the effects of early treatment, infarcted animals were

Table 3 Effect of imidapril treatment for 5 weeks on the left ventricular adenylyl cyclase activity in 3 days infarcted rats.

	Adenylyl cyclase activity (pmol cAMP/mg protein/min)			
	Sham	MI	Sham + IMP	IMP + MI
Basal	30 ± 2.5	20 ± 1.6*	29 ± 1.8	31 ± 2.4 [†]
Forskolin (100 µM)	429 ± 27	302 ± 21*	470 ± 34	460 ± 32 [†]
NaF (5 mm)	245 ± 19	177 ± 15*	265 ± 22	259 ± 23 [†]
Gpp(NH)p (30 µM)	183 ± 12	126 ± 11*	179 ± 14	180 ± 16 [†]

Values are mean ± SE of 6 animals in each group. MI, infarcted animals; IMP, imidapril treated group. * - significantly (P<0.05) different from respective sham; [†] - significantly (P<0.05) different from respective infarcted.

Table 4 Cardiac adenylyl cyclase in the absence and presence of pertussis toxin or cholera toxin in infarcted rats treated with or without imidapril.

	Adenylyl cyclase activity (pmol cAMP/mg/min)			
	Sham	MI	Sham + IMP	IMP + MI
-	37 ± 2.8	19 ± 2.5*	34 ± 2.8	31 ± 1.7†
Pertussis toxin (5 µg/ml)	56 ± 2.1	68 ± 2.8*	53 ± 2.4	54 ± 2.5†
Cholera toxin (20 µg/ml)	71 ± 3.9	28 ± 1.2*	69 ± 3.8	72 ± 4.1†

Values are mean ± SE of 4 experiments in each group. MI, infarcted rats; IMP, imidapril treated animals; * - significantly (P<0.05) different from sham; † - significantly (P<0.05) different from MI group.

treated with imidapril 3 days after infarction and treatment was continued for 5 weeks. Results in Table 3 demonstrate similar changes in basal and stimulated AC activities as seen when imidapril treatment was delayed for 3 weeks. It should be noted that full scar develops after 3 weeks of coronary ligation in this rat model.

The activities of G_s - and G_i -proteins in cardiac membranes were assessed by incubation with pertussis toxin, which stimulates AC by locking G_i - proteins in an inactive form; and cholera toxin, which stimulates adenylyl cyclase by locking G_s -proteins in an active form. Incubation of membranes from the left ventricle with pertussis toxin resulted in an increase in AC activity in infarcted animals when compared to the respective control group (Table 4). Imidapril treatment prevented the increase in AC activity upon pertussis toxin treatment of left ventricle membranes from infarcted animals. On the other hand, incubation of left ventricular membranes with cholera toxin produced a significant decrease in the AC activity in infarcted hearts. Treatment of infarcted animals with imidapril prevented the decrease in AC activity upon incubation of crude membranes from infarcted animals with cholera toxin.

To measure relative amounts of $G_{i\alpha}$ - and $G_{s\alpha}$ -proteins in membranes from left ventricles of control and infarcted hearts, immunoblotting was performed by using antibodies from polyclonal antiserum raised against $G_{i\alpha 2}$ - and $G_{s\alpha}$ -subunits. Results of the densitometric analysis of the immunoblots

Fig. 1 Analysis of immunoblots of G_i - and G_s -protein contents in left ventricles of control, infarcted and imidapril treated failing animals. Each value is a mean ± S.E. of 4-6 experiments. G_i -protein content was measured at 42 kD band and G_s -protein content was from the 52 kD band. *P<0.05, significantly different from sham control. #P<0.05, significantly different from sham + MI.

suggest that while there was an increase in the amount of $G_{i\alpha}$ -proteins in left ventricles from infarcted animals, the levels of G_s -proteins were unaltered in these animals. Treatment of infarcted animals with imidapril prevented the increase in the levels of G_i proteins in crude left ventricle membranes from infarcted animals when compared to respective control groups whereas no changes in levels of G_s -proteins were seen with imidapril treatment.

The steady state mRNA levels for both $G_{i\alpha}$ - and $G_{s\alpha}$ -proteins of the left ventricles were measured by the Northern blot analysis; GAPDH mRNA or 18SRNA were used as internal standards. The densitometric analysis of Northern blots (Fig. 2) indicate that mRNA levels for G_i -proteins in comparison to the GAPDH mRNA were significantly increased in left ventricles from infarcted animals; these alterations in mRNA levels of Gi-proteins in

membranes from infarcted animals were prevented upon treatment with imidapril. The mRNA levels for G_s proteins in comparison to the GAPDH mRNA were not altered in any of the groups (Fig. 2).

Discussion

In this study we have demonstrated a depression in heart function upon coronary artery ligation in rats. Previous observations have indicated attenuated cardiac function in CHF subsequent to myocardial infarction [24,27,28] and depressed heart function has been also reported in other animal models of heart failure [33,34] and failing human heart [35,36]. Since AC serves as the effector of β -AR signal pathway and amplifies heart function by increasing the formation of cAMP, a decrease in the basal AC activity can thus cause decreased accumulation of cAMP, leading to depressed heart function. In this regard, attenuation of basal AC activity in membrane preparations from failing hearts, suggests an intrinsic defect in the cAMP generating capacity of this enzyme. This defect may thus be responsible for the decreased cardiac function in failing animals in our study. It should be noted that the decrease in basal cAMP generation was not due to an increased metabolism of cAMP because of the presence of IBMX, a phosphodiesterase inhibitor, throughout our experiments. Similar decrease in reactivity of this membrane bound enzyme has been found to occur in this [29] and other models of heart failure [37]. Consistent with this hypothesis of an intrinsic defect in the catalytic subunit of AC was the observation that efficacy of forskolin to stimulate AC activity was decreased in the infarcted animals. These results are in agreement with previous findings where both the efficacy and potency of forskolin to stimulate AC was decreased in animal models of heart failure [29,37].

Due to the regulatory roles of G_s - and G_i -proteins in maintaining AC activity, several possibilities exist for the observed decrease in AC leading to depressed cardiac function in the failing hearts. One possibility could be decreased content of G_s -protein. In this regard, in comparison to untreated failing animals, a significant increase in the AC in the

Fig. 2 G-protein mRNA/GAPDH mRNA signal ratio for left ventricles from control, infarcted and imidapril treated failing animals. Each value is a mean $\pm$ S.E. of 4-6 experiments. * $P < 0.05$, significantly different from sham control. # $P < 0.05$, significantly different from sham + MI.

presence of Gpp(NH)p, which is known to affect the enzyme activity through their interaction with G_s-proteins [30], suggests changes in G_s-protein function. Although the activity of G_s-proteins, measured by cholera toxin-stimulated AC activity in failing animals was decreased, G_s-protein content and the level of G_s-protein mRNA in the failing left ventricle were not changed. Thus it is unlikely that the attenuated basal AC activities of failing hearts are due to any changes in the G_s-protein content. The view is consistent with previous findings in which the measurement of Gpp(NH)p-stimulated AC activity upon inhibition of G_i-proteins with pertussis toxin did not increase the functional activity of G_{sα}-proteins [30]. Accordingly, it is suggested that the apparent decrease in functional activity of G_s-proteins in the experimental preparations from the left ventricle may be due to the presence of an excessive amount of G_i-proteins in the membranes. Our observation with respect to increased pertussis toxin stimulated AC activity in the failing hearts due to MI is in agreement with findings in animals [30] and in failing human heart [32]. Such increases in the functional activities associated with the G_i-proteins in the failing heart may be due to increased contents of the G_i-protein per se. In fact, experiments reported in this study, reveal an increase in G_i-protein content in the failing left ventricles. Because the steady state mRNA level for G_i-proteins was increased in the failing left ventricle, it is likely that the increased G_i-protein content may be a consequence of increased gene expression.

It seems that the attenuated AC activity of the left ventricles from failing animals may be due to a defective catalytic activity of AC as well as changes in the content, function and gene expression of G_i-proteins. In fact a direct interaction of GTP-G_{iα} complex with AC type V and VI, the dominant isoforms in cardiac tissue, has been detected by using purified AC and G_{iα} protein in reconstituted membranes [38]. Since the cardiac L-type calcium current is an important regulator of myocardial contractility, it is possible that G_i-protein may decrease this current and then depress the cardiac function. In this regard, it has been shown that G_i-protein is not only a critical component in the muscarinic regulation of inhibiting cardiac L-type Ca²⁺ currents

[39,40], but can also inhibit Ca²⁺-channels directly [41]. Thus, the increased basal level of G_i-protein in the failing heart may depress cardiac function by affecting the L-type calcium channels. Although G_{sα}-protein overexpression alone has also been shown to be sufficient to mediate a signal transduction mechanism, which constitutively enhances L-type Ca²⁺ currents independent of the cAMP pathway [42], this mechanism may not be involved in cardiac depression because no change in the expression of G_s-proteins was observed in the failing heart.

Since the improvement of cardiac function by imidapril was associated with prevention of changes in the catalytic activity of AC as well as the activities and expression of G_i-protein, it is plausible that the improved cardiac function in heart failure upon treatment with imidapril may be due to preservation of the catalytic activities of AC as well as lowering of the G_i-protein content.

Acknowledgements

The work reported in this paper was supported by a grant from the Heart and Stroke Foundation of Manitoba. N.S. Dhalla holds the Canadian Institute of Health Research/Pharmaceutical Research and Development Chair in Cardiovascular Research supported by Merck Frosst Canada. Imidapril was kindly supplied by Tanabe Seiyaku Co., Osaka, Japan.

References

1. **Bristow M.R., Laser J.A., Ginsburg R., Minobe W.**, β₁ and β₂ receptors are coupled to adenylate cyclase in human ventricular myocardium. *Circ. Res.*, **59**:297-308, 1986
2. **Feldman A.M.**, Experimental issues in assessment of G-proteins in cardiac disease, *Circulation*, **84**:1852-1861, 1991
3. **Dhalla N.S., Wang X., Sethi R., Das P.K., Beamish R.E.**, β-adrenergic linked signal mechanisms in failing hearts. *Heart Failure Reviews* **2**:55-65, 1997
4. **Lohse M.J.**, Molecular mechanisms of membrane receptor desensitization. *Biochim. Biophys. Acta.*, **1179**:171-188, 1993
5. **Hammond H.K.**, Mechanisms for myocardial β-adrenergic receptor desensitization in heart failure. *Circulation*, **87**:652-654, 1993

6. **Bohm M., Gierschik P., Knorr A., Larisch K., Weismann K., Erdmann E.**, Desensitization of adenylyl cyclase and increase of Gi in cardiac hypertrophy due to acquired hypertension. *Hypertension*, **20**:103-112, 1992
7. **Marzo K.P., Frey M.J., Wilson J.R., Liang B.T., Manning D.R., Lanoce V., Molinoff P.B.**, β -adrenergic receptor-G-protein-adenylate cyclase complex in experimental canine congestive heart failure produced by rapid ventricular pacing. *Circ. Res.*, **69**:1546-1556, 1991
8. **Chen L.A., Vatner D.E., Vatner S.F., Hittinger L., Homcy C.J.**, Decreased G_s mRNA levels accompany the fall in G_s and adenylyl cyclase activities in compensated left ventricular hypertrophy. In heart failure, only the impairment in adenylyl cyclase activation progresses. *J. Clin. Invest.*, **87**:293-298, 1991
9. **Longabaugh J., Vatner D.E., Vatner S.F., Homcy C.J.**, Decreased stimulatory guanosine triphosphate binding protein in dogs with pressure overload left ventricular failure. *J. Clin. Invest.*, **81**:420-424, 1988
10. **Shi B., Heavner J.E., McMahon K.K., Spallholz J.E.**, Dynamic changes in Gi levels in rat hearts associated with impaired heart function after myocardial infarction. *Am. J. Physiol.*, **269**:H1073-H1079, 1995
11. **Kacimi R., Gerdes A.M.**, Alterations in G protein and MAP kinase signaling pathways during cardiac remodeling in hypertension and heart failure. *Hypertension*, **41**:968-977, 2003
12. **Wieland T., Mittmann C.**, Regulators of G-protein signalling: multifunctional proteins with impact on signalling in the cardiovascular system. *Pharmacol. Ther.*, **97**:95-115, 2003
13. **Esposito G., Rapacciuolo A., Naga Prasad S.V., Rockman H.A.**, Cardiac hypertrophy: role of G protein-coupled receptors. *J. Card. Fail.*, **8**:S409-S414, 2002
14. **Rapacciuolo A., Esposito G., Prasad S.V., Rockman H.A.**, G protein-coupled receptor signalling in vivo cardiac overload. *Acta. Physiol. Scand.*, **173**:51-57, 2001
15. **Bohm M., Kirchmayr R., Erdmann E.**, Myocardial G-protein levels in patients with hypertensive cardiac hypertrophy, ischemic heart disease and cardiogenic shock. *Cardiovasc. Res.*, **30**:611-618, 1995
16. **Feldman A.M., Cates A.E., Bristow M.R., Van D.C.**, Altered expression of α -subunits of G-proteins in failing human hearts. *J. Mol. Cell. Cardiol.*, **21**:359-365, 1989
17. **Wollert K.C., Drexler H.**, The renin-angiotensin system and experimental heart failure. *Cardiovasc. Res.*, **43**:838-849, 1999
18. **Ren B., Lukas A., Shao Q., Guo M., Takeda N., Aitken R.M., Dhalla N.S.**, Electrocardiographic changes and mortality due to myocardial infarction in rats with or without imidapril treatment. *J. Cardiovasc. Pharmacol. Therapeut.*, **3**:11-22, 1998
19. **Kuizinga M.C., Smits J.F., Arends J.W., Daemen M.J.A.P.**, AT2 receptor blockade reduces cardiac interstitial cell DNA synthesis and cardiac function after rat myocardial infarction. *J. Mol. Cell. Cardiol.*, **30**:425-434, 1998
20. **Liu Y-H., Yang X-P., Sharov V.G., Nass O., Sabbah H.N., Peterson E., Carretero O.A.**, Effects of angiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor antagonists in rats with heart failure. Role of kinins and angiotensin II type 2 receptors. *J. Clin. Invest.*, **99**:1926-1935, 1997
21. **Gervais M., Fornes P., Richer C., Nisato D., Giudicelli J.F.**, Effects of angiotensin II AT1-receptor blockade on coronary dynamics, function, and structure in postischemic heart failure in rats. *J. Cardiovasc. Pharmacol.*, **36**:329-337, 2000
22. **Flesh M., Schiffer F., Zolko O., Pinto Y., Stasch J.P., Knorr A., Ettelbruck S., Bohm M.**, Angiotensin receptor antagonism and angiotensin convertin enzyme inhibition improve diastolic dysfunction and Ca^{2+} -ATPase expression in the sarcoplasmic reticulum in hypertensive cardiomyopathy. *J. Hypertension*, **15**:1001-1009, 1997
23. **Shao Q., Ren B., Zarain-Herzberg A., Ganguly P.K., Dhalla N.S.**, Captopril treatment improves the sarcoplasmic reticular Ca^{2+} transport in heart failure due to myocardial infarction. *J. Mol. Cell. Cardiol.*, **31**:1663-1672, 1999
24. **Wang J., Liu X., Ren B., Rupp H., Takeda N., Dhalla N.S.**, Modification of myosin gene expression by imidapril in failing heart due to myocardial infarction. *J. Mol. Cell. Cardiol.*, **34**:847-857, 2002
25. **Tappia P.S., Liu S.Y., Shatadal S., Takeda N., Dhalla N.S., Panagia V.**, Changes in sarcolemmal PLC isoenzymes in postinfarct congestive heart failure: partial correction by imidapril. *Am. J. Physiol.*, **277**:H40-H49, 1999
26. **Wang X., Dhalla N.S.**, Modification of β -adrenoceptor signal transduction pathway by genetic manipulation and heart failure. *Mol. Cell. Biochem.*, **214**:131-155, 2000
27. **Sethi R., Dhalla N.S.**, Inotropic responses to isoproterenol in congestive heart failure subsequent to myocardial infarction in rats. *J. Cardiac Failure*, **5**:391-399, 1995
28. **Wang J., Liu X., Sentex E., Takeda N., Dhalla N.S.**, Increased expression of protein kinase C isoforms in heart failure due to myocardial infarction. *Am. J. Physiol. Heart Circ. Physiol.*, **284**:H2277-H2287, 2003
29. **Sethi R., Dhalla K.S., Beamish R.E., Dhalla N.S.**, Differential changes in left and right ventricular adenylyl-cyclase activities in congestive heart failure. *Am. J. Physiol.*, **272**:H884-H893, 1997
30. **Sethi R., Elimban V., Chapman D., Dixon I.M.C., Dhalla N.S.**, Differential alterations in left and right ventricular G-proteins in congestive heart failure due to myocardial infarction. *J. Mol. Cell. Cardiol.*, **30**:2153-2163, 1998
31. **Choczynski P., Sacchi M.**, Single step methods of RNA isolation by acid guanidinium thiocyanate phenol-chloroform extraction. *Anal. Biochem.*, **162**:156-159, 1987
32. **Feldman A.M.**, Experimental issues in assessment of G-protein function in cardiac disease. *Circulation*, **84**:1852-1861, 1991
33. **Adamson P.B., Vanoli E., Mattera G.G., Germany R., Gagnol J.P., Carminati P., Schwartz P.J.**, Hemodynamic effects of a new inotropic compound, PST-2744, in dogs with chronic ischemic heart failure. *J. Cardiovasc. Pharmacol.*, **42**:169-173, 2003
34. **Elsherif L., Ortines R.V., Saari J.T., Kang Y.J.**, Congestive heart failure in copper-deficient mice. *Exp. Biol. Med. (Maywood)*, **228**:811-817, 2003

35. **Reiken S., Wehrens X.H., Vest J.A., Barbone A., Klotz S., Mancini D., Burkhoff D., Marks A.R.,** Beta-blockers restore calcium release channel function and improve cardiac muscle performance in human heart failure. *Circulation*, **107**:2459-2466, 2003
36. **Sarsero D., Russell F.D., Lynham J.A., Rabnott G., Yang I., Fong K.M., Li L., Kaumann A.J., Molenaar P.,** (-)-CGP 12177 increases contractile force and hastens relaxation of human myocardial preparations through a propranolol-resistant state of the beta 1-adrenoceptor. *Naunyn Schmiedeberg's Arch. Pharmacol.*, **367**:10-21, 2003
37. **Calderone A., Bouvier M., Li K.,** Dysfunction of the beta and alpha adrenergic system in a model of congestive heart failure. The pacing-overdrive dog. *Circ. Res.*, **69**:332-333, 1991
38. **Taussig R., Tang W.J., Hepler J.R., Gilman A.G.,** Distinct patterns of bidirectional regulation of mammalian adenylyl cyclases. *J. Biol. Chem.*, **269**:6093-6100, 1994
39. **Chen F., Spicher K., Jiang M., Birnbaumer L., Wetzel G.T.,** Lack of muscarinic regulation of Ca(2+) channels in G(i2)alpha gene knockout mouse hearts. *Am. J. Physiol. Heart. Circ. Physiol.*, **280**:H1989-1995, 2001
40. **Gilon P., Yakel J., Gromada J., Zhu Y., Henquin J.C., Rorsman P.,** G protein-dependent inhibition of L-type Ca2+ currents by acetylcholine in mouse pancreatic B cells. *J. Physiol.*, **499**:65-76, 1997
41. **Wolfe J.T., Wang H., Howard J., Garrison J.C., Barrett P.Q.,** T-type calcium channel regulation by specific G-protein betagamma subunits. *Nature*, **424**:209-213, 2003
42. **Lader A.S., Xiao Y.F., Ishikawa Y., Cui Y., Vatner D.E., Vatner S.F., Homcy C.J., Cantiello H.F.,** Cardiac Gs alpha overexpression enhances L-type calcium channels through an adenylyl cyclase independent pathway. *Proc. Natl. Acad. Sci., USA*, **95**:9669-9674, 1998