Role of sympathetic cotransmitter galanin on autonomic balance in heart failure: an active player or a bystander?

Demet Özkaramanlı Gür, Miray Sağbaş*, Aydın Akyüz, Savaş Güzel**, Şeref Alpsoy, Niyazi Güler

Departments of Cardiology, *Degree of Science, **Biochemistry, Faculty of Medicine, Namık Kemal University; Tekirdağ-*Turkey*

ABSTRACT

Objective: Galanin, a cotransmitter similar to neuropeptide Y (NPY), aggravates autonomic imbalance in systolic heart failure (HF) by attenuating vagal tonus after burst sympathetic activity. In animal HF models, galanin antagonists have improved cardiac function. To determine whether galanin is a promising therapeutic target in HF, we studied its concentrations in HF patients and evaluated its correlation with NPY, markers of humoral activity such as pro-BNP and copeptin, and echocardiographic parameters of HF severity.

Methods: After recording demographic and echocardiographic characteristics of 87 individuals (57 HF patients and 30 control subjects), fasting serum concentrations of galanin, NPY, copeptin, and pro-BNP were determined.

Results: Unlike pro-BNP, copeptin, and NPY, which were significantly elevated in HF patients (p<0.001, p<0.001, and p=0.001, respectively), galanin was similar in HF patients and control subjects (p=0.9). NPY correlated with the echocardiographic parameters of HF severity (r=–0.22, p=0.03 for EF; r=0.3, p=0.005 for Tei index of RV; r=–0.23, p=0.03 for TAPSE; and r=0.24, p=0.024 for E/e') and pro-BNP (r=0.22, p=0.046). NPY levels were also associated with beta blocker (BB) use, wherein BB significantly decreased NPY in both HF patients and control subjects. Galanin correlated with humoral biomarkers, pro-BNP and copeptin (r=0.39, p<0.001 and r=0.41, p<0.001, respectively). Although current smoking, BB therapy, pro-BNP, copeptin, and body mass index were associated with galanin in univariate analyses, the multiple linear regression model revealed that pro-BNP was the only significant determinant of galanin levels in HF patients.

Conclusion: Our findings confirmed the role of NPY in autonomic balance and suggest that galanin is associated with the proadrenergic state, but its role in HF in humans remains unclear. *(Anatol J Cardiol 2017; 18: 281-88)*

Keywords: heart failure, galanin, neuropeptide Y, sympathovagal crosstalk

Introduction

One of the main disturbances in heart failure (HF) is characterized by autonomic imbalance in favor of the sympathetic nervous system such that both the sympathetic activation and parasympathetic withdrawal contribute to the impaired equilibrium. Current evidence has shown that sympathetic activation also results in parasympathetic withdrawal, demonstrating the so called "sympathovagal crosstalk" (1, 2). Cotransmitters, which are located in the vesicles adjacent to norepinephrine and released in response to high-frequency stimuli, are responsible for such communication. They reduce cardiac vagal function by inhibiting acetylcholine release, further aggravating the imbalance. Neuropeptide Y (NPY) and galanin are the two main neuropeptides postulated to be responsible for sympathovagal crosstalk (3).

NPY has an established role in diseases with sympathetic hyperactivity like hypertension, systolic HF, and acute myocardial infarction, wherein its levels also correlate with mortality (4, 5).

However, the cardiovascular effects of the galanin peptide family, although discovered earlier, still need to be elucidated. Evidence from animal studies demonstrated that it reduces sympathetic vasomotor tone both centrally in the nucleus solitarius and peripherally through stellate ganglia (6, 7). Furthermore, blockage of galanin receptors improved cardiac function and attenuated ventricular remodeling in a rat systolic HF model, making galanin receptors a novel target for treatment (8).

Because most of the evidence pertaining to galanin is derived from animal studies on systolic HF, data on "sympathovagal crosstalk" in humans and that of the cardiovascular role of galanin in the failing human heart are limited. Therefore, we evaluated whether the plasma levels of galanin correlated with NPY levels, the activity of humoral system represented by pro-BNP and copeptin, and myocardial performance estimated by echocardiography in HF patients. To the best of our knowledge, this is the first study to document plasma concentrations of galanin in chronic systolic HF patients.

Methods

This was a prospective case-control study comprising 57 HF patients and 30 control subjects. The power of the study was 80%, with a reliability of 95%. The inclusion criteria were the presence of chronic stage C systolic HF [HF with ejection fraction (EF) of 45% or less] with a New York Heart Association (NYHA) class II or III functional capacity. "Chronicity" was defined as having symptoms of HF without revascularization or cardiac resynchronization therapy within the past 3 months. Patients younger than 18 years and patients with inflammatory disorders, congenital or valvular heart disease, active myocarditis, and primary diastolic HF such as those showing hypertrophic or restrictive cardiomyopathy and chronic kidney disease were excluded. Chronic kidney disease was defined as an estimated glomerular filtration rate (eGFR) of <60 mL/min. Patients without overt HF symptoms showing NYHA class I and those with acutely decompensated symptoms showing NYHA class IV who would require inotropic support were not included. The study was approved by the local Ethics Committee, and its protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Informed consent was obtained from each patient prior to enrollment.

Patient enrollment

Of 81 consecutive systolic HF patients who presented with symptoms of HF between March and August 2016, 57 with characteristics satisfying the inclusion criteria were enrolled in the study. The reason of exclusion was recent coronary revascularization in 5, recent resynchronization device therapy in 3, eGFR<60 mL/min in 9, and no prior coronary angiography to define the etiology in seven patients. The control group comprised volunteers who presented to the cardiology clinic for nonspecific cardiovascular symptoms and in whom detailed cardiovascular evaluation revealed normal systolic and diastolic ventricular functions. Presence of specific cardiac disorders such as coronary artery disease was avoided in the control population.

Age, sex, height, weight, current smoking status, family history of cardiovascular disease, presence of diabetes mellitus (DM), hypertension (HT), or hyperlipidemia (HL), and the medications used were recorded for all subjects at the first medical consultation. The electrocardiographic recordings for determination of rhythm as sinus or atrial fibrillation were obtained for study population. The etiology of HF as either ischemic or nonischemic cardiomyopathy was determined for each patient based on the medical history.

Body weight and height were measured using a digital scale. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Body surface area (BSA) was calculated using the DuBois formula (9).

Echocardiographic measurements

All patients underwent detailed transthoracic echocardiography with General Electric Vivid 6S (GE Healthcare USA) ultrasound machine to determine left and right ventricular functions and left atrial dimensions at the time of presentation. The echocardiographic parameters used to represent myocardial performance and the severity of HF were left atrial volume index (LAVI), left ventricular mass index (LVMI), relative wall thickness (RWT), EF, ratio of mitral E velocity to averaged e' velocities measured at both septal and lateral mitral annuli (E/e'), myocardial performance indices (Tei indices) for both ventricles, and tricuspid annular plane systolic excursion (TAPSE) of the right ventricle.

Left atrial volume was determined using the biplane area–length method and indexed to BSA (10). Left ventricular mass and RWT were calculated using the left ventricular dimensions in the parasternal long axis view and indexed to BSA as described earlier (10). EF was measured using the modified Simpsons biplane method in the apical 4-chamber and 2-chamber views (10). E wave was measured based on the mitral inflow velocities obtained by pulse wave (PW) Doppler in the apical 4-chamber view. Lateral and septal mitral annular early diastolic (e') velocities were also measured by PW Doppler in the apical 4-chamber view, and the average e' was calculated and used in E/e'. Tei indices for both right and left ventricles were determined by PW Doppler as a measure of combined systolic and diastolic ventricular functions (11). TAPSE was measured to determine the RV systolic function using M mode in the apical 4-chamber view.

Blood collection and analysis

Serum concentrations of galanin, NPY, pro-BNP, and copeptin were determined for all subjects at the time of presentation. Galanin and NPY were used as the markers of sympathovagal crosstalk, whereas pro-BNP and copeptin were used as a measure of humoral activity (12).

Following overnight fasting, 5 mL of blood sample was collected from a vein in the antecubital fossa without venous occlusion, and the sample was immediately centrifuged at 3000 rpm for 10 min in refrigerated centrifuge. Serum sample was then stored at −86°C until biochemical analysis. Serum galanin levels were measured by an Elabscience ELISA Kit based on competition principle and microtiter plate separation (Human GAL ELISA Kit Catalog no: E-EL-H1301, Elabscience Biotechnology Co., Ltd., WuHan, PCR). Inter-assay and intra-assay coefficients of variability were <10%. The minimum detectable dose of human galanin was 9.375 pg/mL. Serum NPY levels were measured by an ELISA Kit (Human NPY ELISA Kit Catalog no: E-EL_H1893, Elabscience Biotechnology Co., Ltd., WuHan, PCR). Similarly, the intra-assay and inter-assay variabilities of the ELISA Kit were <10%, and the minimum detectable dose of NPY was 18.75 pg/mL. Serum pro-BNP and copeptin levels were measured by an ELISA Kit (Human pro-BNP ELISA Kit Catalog no: E-EL-H0902 and Human CPP (Copeptin) ELISA Kit Catalog no: E-EL-H0851, Elabscience Biotechnology Co., Ltd., WuHan, PCR). The intra-assay and inter-assay variabilities of the ELISA Kit were <10%. The minimum detectable dose of human pro-BNP was 23.438 pg/mL, and the minimum detectable dose of human copeptin was 18.75 pg/mL.

Anatol J Cardiol 2017; 18: 281-88
DOI:10.14744/AnatolJCardiol.2017.7831

Özkaramanlı Gür et al.
Sympathetic Co-transmitter Galanin   *283*

Table 1. Demographic and echocardiographic characteristics of the study population

	HF patients			Control			*P*
	Mean±SD	Median	f	Mean±SD	Median	f	
Age, years	64±11.3			48.6±11.8			*P*<0.001
Gender, female,%, n	21.1 (12)		21.1 (12)			36.7 (11)	0.12
Current smoker, %, n	7 (4)		7(4)			36.7 (11)	0.01
Family history,%, n	47.4 (27)		47.4 (27)			50 (15)	0.815
Diabetes mellitus, %, n	31.6 (18)		31.6 (18)			16.7 (5)	0.134
Hypertension, %, n	78.9 (45)		78.9 (45)			23.3 (7)	*P*<0.001
Hyperlipidemia, %, n	47.4 (27)		47.4 (27)			36.7 (11)	0.339
BMI, kg/m²	28.1±4.1			29.3±4.5			0.22
BSA, m²	1.9±0.14			1.95±0.19			0.18
NYHA functional class							
II, %, n			89.5 (51)			–	
III, %, n			10.5 (6)			–	
Etiology							
Ischemic, %, n			68.4 (41)			–	
Nonischemic, %, n			31.6 (19)			–	
Sinus rhythm, %, n			81.6 (47)			100 (30)	
LAVI, mL/m²	67.2±20.9	61		28±6.3	28.5		*P*<0.001*
LVMI, g/m²	140.1±36.1	137		88.6±17.4	85		*P*<0.001**
RWT	0.29±0.06	0.28		0.36±0.06	0.36		*P*<0.001**
EF, %	28.9±6.6	28.8		62.5±5.3	62		*P*<0.001**
Tei LV	0.72±0.25	0.71		0.47±0.18	0.43		*P*<0.001*
Tei RV	0.68±0.26	0.59		0.39±0.17	0.31		*P*<0.001*
TAPSE, mm	18±3.5	17		25.6±3.8	25		*P*<0.001*
E/e'	15.1±4.4	17		6.8±1.68	6.7		*P*<0.001
Treatment with, %, n							
Beta adrenergic blockers			82.5 (47)			13.3 (4)	
Digitalis			14(8)			–	
Spironolactone			49.1 (28)			–	
ACE inhibitor			56.1 (32)			–	
ARB			15.8 (9)			3.3 (1)	
Ivabradine			12.3 (7)			–	

BMI - body mass index; BSA - body surface area; EF - ejection fraction; LAVI - left atrial volume index; LV - left ventricle; LVMI - left ventricular mass index; NYHA - New York Heart Association; RWT - relative wall thickness; RV - right ventricle; TAPSE - tricuspid annular plane systolic excursion. *P**: The variables are compared using Mann–Whitney U test; *P***: The variables are compared using independent samples t-test

Statistical methods

Statistical analyses were performed using IBM® SPSS® Statistics for Windows, Version 22 software (IBM Corp., Armonk, NY). Continuous variables are represented as mean±standard deviation (SD) and categorical variables are represented as percentages. The variables were tested for normal distribution using Kolmogorov–Smirnov test. The comparison of patients with control subjects was performed using independent samples t-test for normally distributed variables and Mann–Whitney U test for abnormally distributed variables. The categorical variables were compared using chi-square test. The Pearson and Spearman correlation coefficients were calculated to evaluate continuous and non-continuous relationships among biomarkers and other variables. With setting serum galanin levels as the dependent variable, multiple linear regression analysis was conducted, and the variables with a p value of <0.1 were included into the model by the enter method to determine the predictors of serum galanin levels. Receiver operating characteristic (ROC)

284 Özkaramanlı Gür et al.
Sympathetic Co-transmitter Galanin

Anatol J Cardiol 2017; 18: 281-88
DOI:10.14744/AnatolJCardiol.2017.7831

curves were constructed for biochemical markers to describe their diagnostic properties. A p value of <0.05 was considered statistically significant.

Results

Anthropometric and clinical features

Fifty-seven HF patients (HF group) and 30 control subjects were enrolled into the study. Among HF patients, 68.4% (n=39) had ischemic and 31.6% (n=18) had nonischemic cardiomyopathy. All control subjects and most HF patients (82.5%, n=47) were in sinus rhythm. The demographic and echocardiographic characteristics of the patients and control subjects are presented in Table 1.

Echocardiographic features

Patients with HF had higher LAVI [67.2±20.9 (61) vs. 28±6.3 (28.5) mL/m², p<0.001], LVMI [140.1±36.1 (137) vs. 88.6±17.4 (85) g/m², p<0.001], E/e′ [15.1±4.4 (13.8) vs. 6.8±1.68 (6.7), p<0.001], and left and right ventricular Tei indices [0.72±0.25 (0.71) vs.

0.47±0.18 (0.43), p<0.001 and 0.68±0.26 (0.59) vs. 0.39±0.17 (0.31), p<0.001, respectively] as expected. Likewise, EF [28.9±6.6 (28.8) vs. 62.5±5.3 (62), p<0.001], RWT [0.29±0.06 (0.28) vs. 0.36±0.06 (0.36), p<0.001], and TAPSE [18±3.5 (17) vs. 25.6±3.8 (25) mm, p<0.001] were significantly lower in HF patients than those in control subjects.

Biochemical parameters

Table 2 shows the comparison of biochemical parameters among patients with and without HF. As the objectified biomarker of HF, pro-BNP was higher in HF patients than in control subjects (2128.9±1104.5 vs. 212.6±96.4 pg/mL, p<0.001). NPY and copeptin levels were also higher in patients with HF (872.3±280.7 vs. 640.7±279 pg/mL, p<0.001 for NPY and 139.8±65.5 vs. 79.8±35.9 pg/mL, p<0.001 for copeptin), but there was no significant difference in the levels of galanin in patients with and without HF (32.5±19.06 vs. 31.9±18.4 pg/mL, p=0.9).

Table 3 shows the correlations of plasma levels of biochemical markers and echocardiographic and demographic character-

Table 2. Comparison of biochemical markers between HF patients and control subjects

	HF patients		Control group		P
	Mean±SD	Median (min-max)	Mean±SD	Median (min-max)	
NPY, pg/mL	872.3±280.7	914 (181.5–1340.4)	640.7±279	600 (173.4–1033.4)	0.001**
Galanin, pg/mL	32.5±19.06	30	31.9±18.4	29.4 (1.76–72.5)	0.9*
Pro-BNP, pg/mL	2128.9±1104.5	2140	212.6±96.4	219.1 (65–414.4)	P<0.001*
Copeptin, pg/mL	139.8±65.5	133.7	79.8±35.9	76.5 (29.5–159.2)	P<0.001*

HF - heart failure; NPY - neuropeptide Y; Pro-BNP - pro-brain natriuretic peptide. *Independent samples t-test; **Mann–Whitney U test

Table 3. Correlation analysis between biochemical variables and echocardiographic/demographic data

	Pro-BNP		Copeptin		Neuropeptide Y		Galanin	
	r	P	r	P	r	P	r	P
Pro-BNP	1	1	0.69	<0.001	**0.22**	**0.046**	**0.39**	**<0.001**
Copeptin	0.69	<0.001	1	1	0.09	0.42	**0.41**	**<0.001**
NPY	0.22	0.046	0.088	0.416	1	1	-0.122	0.26
Galanin	0.39	<0.001	0.41	<0.001	-0.122	0.261	1	1
LVMI	0.64	<0.001	0.45	<0.001	0.159	0.141	0.017	0.874
LAVI	0.66*	0.008	0.4*	<0.001	0.3*	0.05	0.008	0.943
RWT	0.45*	<0.001	0.25*	0.02	0.159	0.572	-0.095	0.381
EF	-0.71	<0.001	-0.43	<0.001	**-0.22**	**0.03**	0.002	0.987
Tei left	0.43*	<0.001	0.34*	0.001	0.185	0.086	-0.085	0.43
Tei right	0.46*	<0.001	0.26*	0.01	**0.30***	**0.005**	-0.03	0.782
TAPSE	-0.55*	<0.001	-0.25*	0.01	**-0.23***	**0.03**	0.026	0.808
E/e′	0.68*	<0.001	0.43*	<0.001	**0.24***	**0.024**	0.009	0.93
BMI	-0.17	0.12	0.25	0.01	-0.08	0.465	**0.24**	**<0.001**
Age	0.33	0.002	0.21	0.056	**0.3**	**0.005**	-0.011	0.92

r: Pearson coefficient of correlation; *: Spearman coefficient of correlation. BMI - body mass index; LAVI - left atrial volume index; LVMI - left ventricular mass index; RWT - relative wall thickness; NPY - neuropeptide Y; TAPSE - tricuspid annular plane systolic excursion
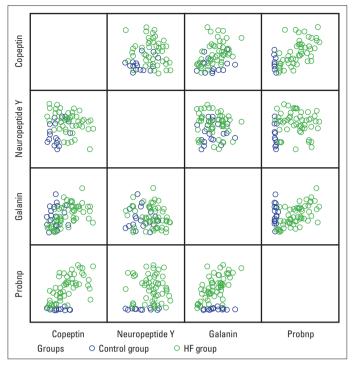
Anatol J Cardiol 2017; 18: 281-88
DOI:10.14744/AnatolJCardiol.2017.7831

Özkaramanlı Gür et al.
Sympathetic Co-transmitter Galanin *285*

Figure 1. Correlation analysis of biomarkers in HF patients (green circles) and control subjects (blue circles) separately

Figure 2. ROC analysis showing biochemical markers in HF

Variable	AUC	SE	*P* value	95% Confidence Interval	
				Lower bound	Upper bound
pro-BNP	.987	.011	.000	.966	1.000
Copeptin	.785	.049	.000	.689	.881
NPY	.715	.056	.001	.604	.826
Galanin	.503	.066	.964	.374	.632

istics of the patients. NPY levels were correlated with pro-BNP (r=0.22, p=0.046), EF (r=–0.22, p=0.03), LAVI (r=0.3, p=0.05), Tei index of RV (r=0.3, p=0.005), TAPSE (r=–0.23, p=0.03), E/e′ (r=0.24, p=0.024), and age (r=0.3, p=0.005). Plasma concentrations of galanin were correlated with humoral biomarkers such as pro-BNP and copeptin (r=0.39, p<0.001 and r=0.41, p<0.001, respectively) but not with any of the echocardiographic parameters of disease severity. There was also a weak correlation between galanin and BMI (r=0.24, p<0.001). Figure 1 shows the correlation analysis of biomarkers in HF patients (green circles) and control subjects (blue circles) separately.

ROC analysis demonstrated that of the four biomarkers evaluated in this study, pro-BNP and copeptin were the most powerful determinants of HF and that galanin had no clinical value (Fig. 2).

Detailed analysis of plasma galanin concentrations and patient characteristics showed that although galanin concentration was similar in HF patients and control subjects, it was higher in currently smoking patients than in nonsmoking controls (51.2±10 vs. 31.9±20.4, p=0.033) or nonsmoking patients (51.2±10 vs. 31.1±18.9, p=0.017) (Table 4). There was no statistically significant difference in galanin concentration in control subjects with or without beta blocker (BB) use, but it was higher in HF patients on BB therapy than in those not on the therapy (p=0.029) (Table 4). To examine the relationship between plasma galanin and specified potential predictors such as age, pro-BNP, copeptin, BMI, current smoking, and BB use, multiple linear regression analysis was conducted in which the model explained a significant amount of variance in galanin levels [R=0.733,
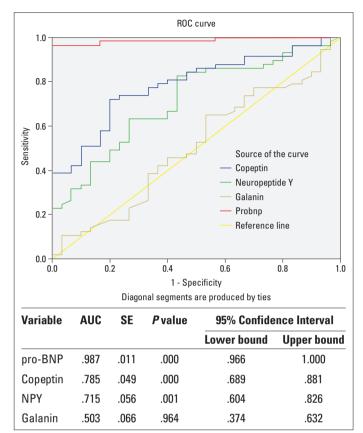
R^2=0.537, F (6,50)=9.653, p<0.001]. After controlling for variables such as current smoking, BB therapy, pro-BNP, copeptin, and BMI in the model, only pro-BNP was a significant predictor of galanin levels.

Subgroup analysis comparing NPY concentrations between patients and control subjects showed that NPY levels were significantly higher in HF patients (Table 4). Analyses of NPY levels within HF patients revealed that NPY was higher in patients without HT (1074±187.9 pg/dL, p=0.004) and in those not on BB therapy (1048±245.1 pg/dL, p=0.027).

Discussion

The present study aimed to answer the question if the plasma levels of galanin correlated with NPY, the classical biomarkers of humoral activity such as pro-BNP and copeptin, or echocardiographic parameters of disease severity in HF patients. We clearly demonstrated that unlike pro-BNP, copeptin, and NPY, galanin was not elevated in chronic HF patients. Galanin was not correlated with echocardiographic parameters of HF severity either. Nevertheless, it was correlated with pro-BNP and copeptin such that after adjusting for other potential predictors, pro-BNP was the only significant determinant of galanin levels in systolic HF patients. Another notable finding in this study was the performance of NPY in determining the efficacy of BB therapy in inhibiting sympathetic activity.

Özkaramanlı Gür et al.
Sympathetic Co-transmitter Galanin

Table 4. Analyses of galanin and NPY levels in patient subgroups of different demographic characteristics

Demographic variables	Subgroups	HF group			Control group			P1	P2
		n	Galanin	NPY	n	Galanin	NPY		
Beta blocker (BB)	BB+	47	34.5±19.8	834.7±275.6	4	24.9±20.0	690.2±293.5	0.13	0.4
	BB-	10	23.3±12.0	1048.8±245.1	26	33.01±18.4	633.1±271.1	0.35	P<0.001
	P**		**0.029**	0.032		0.42	0.8		
Gender	Male	45	32.6±19.1	852.4±297.8	19	34.9±18.1	659.1±284.2	0.66	**0.041**
	Female	12	32.0±19.7	947.1±196.6	11	26.9±18.7	609±253.9	0.52	0.003
	P**		0.92	0.49		0.26	0.60		
Current smoker (CS)	CS+	4	51.2±10	785.3±263.7	11	31.9±20.4	659.2±230.9	**0.033**	0.41
	CS-	53	31.1±18.9	878.9±293.2	19	31.9±17.8	630±296	0.86	**0.005**
	P**		**0.017**	0.55		0.99	0.83		
Family history (FH)	FH+	27	35.66±25.6	898.1±250.2	15	28.8±19.9	653.8±240	0.3	**0.007**
	FH-	30	29.7±17.8	849.1±308	15	35.07±16.9	627.6±305.1	0.33	**0.036**
	P**		0.24	0.58		0.36	0.8		
Diabetes melllitus (DM)	DM+	18	38.5±19.2	868.9±286.9	5	40±13.9	608.4±223.6	0.87	**0.06**
	DM-	39	29.8±18.6	873.9±281.6	25	30.3±19.1	647.2±282	0.9	**0.005**
	P**		0.29	0.85		0.11	0.78		
Hypertension (HT)	HT+	45	34.2±20.3	818.5±278.2	7	33.65±24.3	772.2±229.7	0.94	0.64
	HT-	12	26.0±11.8	1074±187.9	23	31.4±16.9	600.7±273.1	0.33	P<0.001
	P**		0.19	0.006		0.79	0.14		
Hyperlipidemia (HL)	HL+	27	32.4±21.4	870.7±297.5	11	30.7±17	703.9±249.5	0.75	**0.04**
	HL-	29	32.0±17.1	873.7±268.8	19	32.7±19.6	604.1±281.2	0.9	**0.006**
	P**		0.93	0.58		0.78	0.47		
Etiology	Ischemic	39	30.8±20.1	870.4±296.			–		
	Non-ischemic	18	36.2±16.5	876.5±251.6			–		
	P**		0.32	0.58					
Rhythm	Sinus	47	33±19.4	864.1±292.3	30	31.9±18.4	640.7±270.1	0.81	**0.003**
	AF	10	30.2±18	910.7±227		–	–		
	P**		0.67	0.72					
NYHA	2	38	32.8±17.8	914.1±264.7			–		
	3	4	22.3±18.1	669.2±412			–		
	P**		0.27	0.18					

The data is analyzed by independent samples t-test. P1 - Comparison of HF patients and control subjects in terms of subgroups for galanin; P2 - Comparison of HF patients and control subjects in terms of subgroups for NPY; P** - Comparison of subgroups within the HF patients or within control subjects

Neurohumoral activity in HF

HF is a progressive disease mediated by the sustained activation of neurohumoral systems. In our study, we evaluated the plasma levels of copeptin and pro-BNP as markers of humoral activation. Copeptin is a stress hormone, which is released with pre-provasopressin, a precursor of vasopressin, and represents the activity of arginine–vasopressin system (12). The results of copeptin and pro-BNP, the two major biomarkers with established utility in clinical practice, were concordant with the literature and correlated well with HF severity. ROC analysis also demonstrated the efficacy of these biomarkers.

Sympathovagal crosstalk in HF

"Sympathovagal crosstalk," mediated by cotransmitters such as NPY and galanin, explains long-lasting impairment in vagal tone after burst sympathetic stimulation (3). This phenomenon is shown to retain its effect even in the presence of beta adrenergic blockade (3). For HF patients in whom sympathetic hyperactivity is part of the vicious cycle, inhibition of sympathovagal crosstalk represents a putative therapeutic target and is addressed in recent animal studies.

In our study, NPY levels were increased in HF and correlated with pro-BNP, the echocardiographic markers of HF such as EF,

Tei index of RV, TAPSE, and E/e'. Our findings not only confirm the previously reported association of NPY with HF but also demonstrate the relationship of NPY with echocardiographic parameters of disease severity (13, 14).

HF patients who did not receive BB therapy had higher levels of NPY when compared with control subjects or HF patients who received BB therapy, but there was no significant difference between patients and control subjects who received BB therapy. In other words, BB therapy decreased sympathetic activity expressed by NPY levels in HF patients to the levels observed in control subjects who received BB therapy. In contrast to the literature on increased NPY levels in HT, NPY levels in hypertensive HF patients were significantly lower in our study population (15). This seems to reflect the effect of BB because 82.5% of HF patients were already on BB therapy. Therefore, our findings suggest that NPY concentration is a good index of sympathetic activity, and its subsequent decrease reflects the efficacy of BB therapy.

Galanin, a 30 amino-acid peptide, which was discovered prior to NPY, also has deleterious cardiovascular actions resembling those of NPY. Nevertheless, it has a wide range of physiological nonneural actions such as regulation of food intake, energy metabolism, osmotic homeostasis, and inflammation (7). Plasma galanin is demonstrated to increase in patients with type 2 DM, obesity, gestational diabetes, wherein it was shown to reduce insulin resistance (2, 16). Its involvement in both physiological and pathological processes and a variety of its actions are related to its different G protein-coupled receptor subtypes. This challenges the precise definition of its role in cardiovascular disease processes (2).

Our study is the first to evaluate galanin levels in systolic HF patients. In contrast to the animal studies suggesting a close relationship, we failed to demonstrate increased galanin levels in chronic HF patients. Unlike NPY levels, galanin levels were similar in patients and control subjects for all patient subgroups. Although deviations in galanin levels were not concordant with those in NPY levels, this was consistent with the literature. Previous research has proved that administration of galanin in humans decreased vagal tonus, but when galanin levels were studied in response to sympathetic stressors, plasma galanin was not shown to increase (17, 18). This suggests that galanin is not the "the pivot" of autonomic control in a failing human heart. In addition, the serum concentrations of NPY and galanin in the control group were comparable to the control levels in previous studies.

When interpreting the peripheral galanin concentrations, it is also worth noting that galanin is a slowly diffusing peptide, with a very short (5 min) half-life and rapid metabolism and is released in lower concentrations when compared with NPY (2, 19). Thus, its serum levels may not represent the local concentrations in central or cardiac vagal neurons. Our findings together with the previously published data translate that galanin has paracrine modulatory functions on peripheral cardiac sympathetic nerves and failure to demonstrate an elevation in circulating galanin does not eliminate its involvement in sympathovagal crosstalk.

A question raised by this study is why was galanin not elevated in HF patients but was still correlated with pro-BNP and copeptin. The answer lies in the fact that besides being a marker of HF severity, pro-BNP has documented direct proadrenergic features (20). Chan et al. (21) have previously reported that BNP promotes noradrenaline release from cardiac sympathetic nerves. This explains the reason why pro-BNP is positively correlated with both NPY and galanin. The association of pro-BNP with galanin and NPY represents an example of direct interaction, irrespective of HF severity.

The correlation between galanin and copeptin can be explained by galanin's involvement in the central maintenance of energy and osmotic homeostasis (22, 23). Meister et al. (22) have shown that galanin is coexpressed with vasopressin in the central nervous system and that they increase in parallel in cases of increased plasma osmolality.

There are many studies addressing the restoration of autonomic imbalance in HF with either medical or device therapies. Although animal studies of drugs targeting NPY and galanin receptors are promising, clinical translation of experimental data is challenging. Vagal nerve stimulation and carotid baroreflex activation therapies are examples of device therapies with disappointing results (24, 25). The failure of recent clinical trials proved that the autonomic nervous system is not an easy target.

Study limitations

Our study population was relatively small, mainly comprising individuals on chronic BB therapy and showing NYHA class II functional capacity; thus, sympathetic activity may be inadequate to reveal the difference in serum galanin.

Conclusion

In our study, increased NPY levels in HF patients and its subsequent decrease with BB therapy proved the role of NPY in sympathovagal crosstalk. Although we could not provide evidence of increased serum galanin in HF patients, its association with pro-BNP was shown, suggesting an involvement with the proadrenergic state. It can be concluded that dedicated research is needed to define the role of galanin pertaining to the autonomic balance in a failing human heart.

288 Özkaramanlı Gür et al.
Sympathetic Co-transmitter Galanin

Anatol J Cardiol 2017; 18: 281-88
DOI:10.14744/AnatolJCardiol.2017.7831

D.G., M.S., S.G., Ş.A., A.A., N.G.; Writing – D.G., M.S., S.G., Ş.A., A.A., N.G.; Critical review – D.G., M.S., S.G., Ş.A., A.A., N.G.

References

1. Potter E. Cardiovascular actions of galanin. Ann N Y Acad Sci 1998; 863: 170-4.

2. Lang R, Gundlach AL, Holmes FE, Hobson SA, Wynick D, Hokfelt T, et al. Physiology, signaling, and pharmacology of galanin peptides and receptors: three decades of emerging diversity. Pharmacol Rev 2015; 67: 118-75.

3. Shanks J, Herring N. Peripheral cardiac sympathetic hyperactivity in cardiovascular disease: role of neuropeptides. Am J Physiol Regul Integr Comp Physiol 2013; 305: 1411-20.

4. Herring N. Autonomic control of the heart: going beyond the classical neurotransmitters. Exp Physiol 2015; 100: 354-8.

5. Ullman B, Hulting J, Lundberg JM. Prognostic value of plasma neuropeptide-Y in coronary care unit patients with and without acute myocardial infarction. Eur Heart J 1994; 15: 454-61.

6. Díaz-Cabiale Z, Parrado C, Narváez M, Millón C, Puigcerver A, Fuxe K, et al. Neurochemical modulation of central cardiovascular control: the integrative role of galanin. EXS 2010; 102: 113-31.

7. Herring N, Cranley J, Lokale MN, Li D, Shanks J, Alston EN, et al. The cardiac sympathetic co-transmitter galanin reduces acetylcholine release and vagal bradycardia: implications for neural control of cardiac excitability. J Mol Cell Cardiol 2012; 52: 667-76.

8. Chen A, Li M, Song L, Zhang Y, Luo Z, Zhang W, et al. Effects of the Galanin Receptor Antagonist M40 on Cardiac Function and Remodeling in Rats with heart failure. Cardiovasc Ther 2015; 33: 288-93.

9. Du Bois D, Du Bois EF. A formula to estimate the approximate surface area if height and weight be known. 1916. Nutrition 1989; 5: 303-11.

10. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging 2015; 16: 233-70.

11. Tei C, Ling LH, Hodge DO, Bailey KR, Oh JK, Rodeheffer RJ, et al. New index of combined systolic and diastolic myocardial performance: a simple and reproducible measure of cardiac function--a study in normals and dilated cardiomyopathy. J Cardiol 1995; 26: 357-66.

12. Pozsonyi Z, Förhécz Z, Gombos T, Karádi I, Jánoskuti L, Prohászka Z. Copeptin (C-terminal pro arginine-vasopressin) is an independent long-term prognostic marker in heart failure with reduced ejection fraction. Heart Lung Circ 2015; 24: 359-67.

13. Maisel AS, Scott NA, Motulsky HJ, Michel MC, Boublik JH, Rivier JE, et al. Elevation of plasma neuropeptide Y levels in congestive heart failure. Am J Med 1989; 86: 43-8.

14. McDermott BJ, Bell D. NPY and cardiac diseases. Curr Top Med Chem 2007; 7: 1692-703.

15. Baltazi M, Katsiki N, Savopoulos C, Iliadis F, Koliakos G, Hatzitolios AI. Plasma neuropeptide Y (NPY) and alpha-melanocyte stimulating hormone (a-MSH) levels in patients with or without hypertension and/or obesity: a pilot study. Am J Cardiovasc Dis 2011; 1: 48-59.

16. Fang P, Shi M, Zhu Y, Bo P, Zhang Z. Type 2 diabetes mellitus as a disorder of galanin resistance. Exp Gerontol 2016; 73: 72-7.

17. Carey DG, Iismaa TP, Ho KY, Rajkovic IA, Kelly J, Kraegen EW, et al. Potent effects of human galanin in man: growth hormone secretion and vagal blockade. J Clin Endocrinol Metab 1993; 77: 90-3.

18. Ceresini G, Fabbo A, Freddi M, Maggio M, Reali N, Troglio G, et al. Concomitant evaluation of plasma galanin and catecholamine levels during a cold pressor test in healthy human male and female subjects. Horm Res 1997; 48: 268-73.

19. Holmes FE, Bacon A, Pope RJ, Vanderplank PA, Kerr NC, Sukumaran M, et al. Transgenic overexpression of galanin in the dorsal root ganglia modulates pain-related behavior. Proc Natl Acad Sci USA 2003; 100: 6180-5.

20. Thireau J, Karam S, Fauconnier J, Roberge S, Cassan C, Cazorla O, et al. Functional evidence for an active role of B-type natriuretic peptide in cardiac remodelling and pro-arrhythmogenicity. Cardiovasc Res 2012; 95: 59-68.

21. Chan NY, Robador PA, Levi R. Natriuretic peptide-induced catecholamine release from cardiac sympathetic neurons: inhibition by histamine H3 and H4 receptor activation. J Pharmacol Exp Ther 2012; 343: 568-77.

22. Meister B, Cortes R, Villar MJ, Schalling M, Hökfelt T. Peptides and transmitter enzymes in hypothalamic magnocellular neurons after administration of hyperosmotic stimuli: comparison between messenger RNA and peptide/protein levels. Cell Tissue Res 1990; 260: 279-97.

23. Bundzikova J, Pirnik Z, Zelena D, Mikkelsen JD, Kiss A. Response of Substances Co-Expressed in Hypothalamic Magnocellular Neurons to Osmotic Challenges in Normal and Brattleboro Rats. Cell Mol Neurobiol 2008; 28: 1033-47.

24. Zhang Y, Chen A, Song L, Li M, Luo Z, Zhang W, et al. Low-Level Vagus Nerve Stimulation Reverses Cardiac Dysfunction and Subcellular Calcium Handling in Rats With Post-Myocardial Infarction Heart Failure. Int Heart J 2016; 57:350-5.

25. Gold MR, Van Veldhuisen DJ, Hauptman PJ, Borggrefe M, Kubo SH, Lieberman RA, et al. Vagus Nerve Stimulation for the Treatment of Heart Failure: The INOVATE-HF Trial. J Am Coll Cardiol 2016; 68: 149-58.