Experimental Studies

Increased Circulating Calcitonin Gene-Related Peptide in Congestive Heart Failure Caused by Congenital Heart Disease

Jong-Hau Hsu,[1] MD, Jwu-Lai Yeh,[2] PhD, Zen-Kong Dai,[1,3] MD,
Ing-Jun Chen,[2] PhD, *and* Jiunn-Ren Wu,[1,3] MD

SUMMARY

Calcitonin gene-related peptide has potent vasodilatory and inotropic actions. The aim of this study was to characterize the changes in this peptide in children with varying degrees of heart failure secondary to congenital heart disease with left to right shunt and to assess its relationship to systolic pulmonary arterial pressure.

Plasma calcitonin gene-related peptide levels were measured in 131 children including 13 healthy ones, 43 with various degrees of heart failure secondary to congenital heart disease, and 75 with congenital heart disease without heart failure.

In patients with heart failure, calcitonin gene-related peptide concentrations were markedly elevated (15.8 ± 2.1 pg/mL) as compared with healthy control subjects (7.0 ± 0.8 pg/mL, $P < 0.05$) or patients with congenital heart disease but without heart failure (18.6 ± 1.2 pg/mL, $P < 0.01$). Compared with the controls, there were highly significant stepwise increases in the calcitonin gene-related peptide levels in the mild ($n = 15$), moderate ($n = 12$), and severe ($n = 16$) heart failure subgroups by 1.5, 1.7 and 3.4 fold, respectively. The plasma calcitonin gene-related peptide levels also correlated directly with the pulmonary arterial systolic pressure ($r = 0.515$, $P < 0.0001$).

The results of this study indicate that congestive heart failure secondary to congenital heart disease with increased pulmonary flow is associated with elevated levels of calcitonin gene-related peptide that are related to disease severity. Pulmonary overcirculation may play a role in upregulation of calcitonin gene-related peptide in congestive heart failure. (Int Heart J 2005; 46: 867-875)

Key words: Calcitonin gene-related peptide, Congenital heart disease, Heart failure

CALCITONIN gene-related peptide (CGRP), a 37 amino acid residue neurotransmitter peptide derived from the calcitonin gene, is widely distributed in the nervous and cardiovascular systems.[1-4] CGRP has positive inotropic and chronotropic effects on the heart and is also considered the most potent endogenous pep-

tide vasodilator to date.[5-8] Previous reports on the changes of CGRP in congestive heart failure (CHF) are conflicting and the regulation of CGRP production is poorly understood. In adults with CHF secondary to impaired systolic function, the plasma concentration of CGRP has been reported to be unaffected, decreased, or increased.[9-11] In children with congenital heart disease (CHD), CHF is often caused by pulmonary overcirculation. Changes in plasma CGRP levels in CHF secondary to CHD with increased pulmonary flow have not been previously demonstrated.

In order to evaluate the effect of pulmonary blood flow on plasma CGRP levels and the possible role of CGRP in the pathophysiology of CHF caused by CHD, the objectives of this study were: (1) to characterize the changes in circulating CGRP levels in children with varying degrees of CHF secondary to CHD with left to right shunt and (2) to assess the relationship of the CGRP levels to systolic pulmonary arterial pressure.

METHODS

Subjects: We performed a prospectively designed assessment of clinical and laboratory parameters in 118 pediatric patients (55 males, 63 females) with CHD and 13 healthy control subjects. Written informed consent was obtained from the parents of all children prior to the study and the investigation conformed with the principles outlined in the Declaration of Helsinki. The project was approved by the local scientific ethics committee.

Control subjects classified as group A were children with functional heart murmurs. Patients with CHD were divided into two groups according to the presence or absence of CHF. Group B consisted of 75 patients with left to right shunt CHD but no CHF, including 33 with ventricular septal defect (VSD), 27 with atrial septal defect (ASD), 9 with patent ductus arteriosus (PDA), 3 with atrioventricular septal defect, and 3 with ASD and VSD. Group C was made up of 43 patients with CHF due to CHD with left to right shunt, including 25 with VSD, 7 with ASD, 5 with atrioventricular septal defect, 3 with PDA, and 3 with VSD and PDA. No patient in either group B or C received any operation or catheter intervention at the time of recruitment. The degrees of heart failure were estimated at the time of blood sampling, before further intervention. According to the clinical severity of heart failure in infants and children classified by Ross, *et al*[12] the patients in group C were further divided into three groups. Group CI, including 15 individuals with mild tachypnea or dyspnea on exertion, represented mild CHF. Group CII, comprising 12 patients with growth failure and prominent dyspnea on exertion, represented moderate CHF. Group CIII, consisting of 16 patients with chest retraction and diaphoresis at rest, represented severe CHF.

At recruitment, all subjects had their age, weight, height, heart rate, hemogram, and blood pressure recorded. Transthoracic echocardiography was performed in all subjects to record the left ventricular ejection fraction and percentage of fractional shortening. Systolic pulmonary arterial pressure was also measured in Doppler cardiography by extrapolation (modified Bernoulli equation) from the maximum pressure gradient in tricuspid regurgitation,[13] or from the pressure gradient between the left to right shunt if no tricuspid regurgitation was found.[14] Chest radiographs were taken in all subjects except for group A and interpreted by three experienced pediatric cardiologists to determine if there was increased pulmonary vascular markings. The clinical and hemodynamic characteristics of the study subjects are listed in Table I and Table II, respectively.

Blood sample analysis: CGRP was measured by RIA using previously described procedures.[15] Samples for CGRP assays were obtained from the venous blood from each fasting subject after a 20-minute rest in the supine position. All of the

Table I.   Clinical Characteristics of the Study Groups

Group of patients (n)	Gender (M/F)	Age (year)	BW (kg)	Height (cm)	Hb (g/dL)	Hct (%)	PLT ($10^3/\mu$L)
Group A (13)	6/7	5.9 ± 1.3	20.1 ± 2.3	110.4 ± 11.4	12.3 ± 0.8	36.3 ± 2.4	267.2 ± 25.3
Group B (75)	35/40	5.5 ± 1.5	18.2 ± 3.4	103.8 ± 15.2	11.3 ± 1.1	34.5 ± 2.6	283.5 ± 27.1
Group C (43)	20/23	4.8 ± 1.1	14.8 ± 3.7	88.4 ± 13.6	12.5 ± 1.3	35.7 ± 2.0	322.6 ± 28.1
C-I (15)	6/9	5.5 ± 2.7	16.4 ± 3.2	94.8 ± 16.8	12.1 ± 0.9	35.1 ± 2.8	310.0 ± 25.1
C-II (12)	5/7	3.8 ± 1.7	12.8 ± 2.3	85.4 ± 10.2	12.9 ± 1.1	35.9 ± 3.3	326.0 ± 35.2
C-III (16)	9/7	5.1 ± 2.4	14.7 ± 2.7	84.7 ± 13.3	12.5 ± 0.8	36.1 ± 3.1	331.8 ± 28.1

M indicates male; F, female; BW, body weight; Hb, hemoglobin; Hct, hematocrit; and PLT, platelet. Values are mean ± SEM.

Table II.   Hemodynamic Characteristics of the Study Groups

Group of patients (n)	Pulse (min^{-1})	SBP (mmHg)	DBP (mmHg)	EF (%)	FS (%)	PASP (mmHg)	PVM increase (%)
Group A (13)	83.1 ± 8.1	105.2 ± 9.1	70.4 ± 8.1	74.2 ± 2.5	41.8 ± 0.8	21.8 ± 2.4	-
Group B (75)	87.5 ± 9.2	102.2 ± 7.5	67.7 ± 7.9	69.6 ± 7.7	38.8 ± 2.6	24.7 ± 2.7	13 (17.3%)
Group C (43)	91.4 ± 8.3	99.8 ± 7.8	65.3 ± 7.4	75.7 ± 5.5	39.4 ± 3.8	47.3 ± 3.8*#	26 (60.5%)**
C-I (15)	88.6 ± 7.8	100.2 ± 8.5	66.1 ± 5.1	74.6 ± 4.2	39.3 ± 3.1	39.7 ± 4.9	6 (40.0%)
C-II (12)	90.5 ± 7.5	95.2 ± 5.6	62.7 ± 7.2	77.0 ± 5.4	40.8 ± 2.7	44.6 ± 5.7	8 (66.7%)
C-III (16)	94.7 ± 7.1	102.8 ± 7.3	66.5 ± 8.4	75.7 ± 5.6	38.4 ± 3.2	56.3 ± 6.9	12 (75.0%)†

EF indicates ejection fraction; FS, fractional shortening; PASP, pulmonary arterial systolic pressure; and SBP, systolic blood pressure. PVM increase (%), number and percentage of patients with pulmonary vascular markings (PVM) increase. Values are mean ± SEM. *$P < 0.001$ versus group A; #$P < 0.01$ versus group B; **$P < 0.0001$ versus group B,
†$P < 0.05$ versus group C-I.

samples were collected into blood bottles containing EDTA + aprotinin and then placed immediately on ice. After centrifugation, the supernatant was collected and stored at -80°C for further analysis.

Plasma levels of CGRP were measured using a commercially available radioimmunoassay kit, as per instructions (RIK 6009, Peninsula Laboratories, USA). Plasma samples for CGRP analysis were extracted using Sep-pak C-18 cartridges (Waters, USA). The column was preconditioned with 1 mL buffer B [60% acetonitrile in 1% trifluoroacetic acid (TFA) followed by three washes with 3 mL buffer A (1% TFA)]. Acidified plasma (1 mL plasma acidified with 1 mL of 1% TFA) was loaded onto the conditioned column and allowed to pass without applying any pressure. The column was then washed two times with 3 mL of buffer A. Bound CGRP was eluted from the column with 3 mL of buffer B and collected into polypropylene tubes. The eluate was evaporated to dryness under a centrifugal concentrator.

Each residue was reconstituted with 250 μL of assay buffer. Reconstituted extract (100 μL) was incubated with 100 μL of antiserum at 4°C for 24 hours. Following this preincubation, 100 μL of radiolabelled CGRP was added and the assay incubated for a further 24 hours at 4°C. Separation was effected by the addition of 100 μL of goat anti-rabbit IgG serum, followed by 100 μL of normal rabbit serum. Samples were incubated at room temperature for 90 minutes. Assay buffer (0.5 mL) was added to each tube and the tube was centrifuged at 3000 rpm for 20 minutes at 4°C. The supernatant was aspirated and the radioactivity in each tube determined by counting for 120 seconds on a gamma counter. The sensitivity of the assay was 1 pg/mL and the intra- and interassay coefficients of variation were 6 and 9%, respectively. All of the samples were assayed in one batch.

Statistics: All results are reported as the mean ± SEM. Groups were statistically analyzed using the unpaired Student's *t*-test or ANOVA, except for the percentage of patients with increased radiographic lung markings, which was analyzed by the Chi-square test. Simple linear regression analysis was performed to correlate CGRP and systolic pulmonary arterial pressure. A *P* value of less than 0.05 was considered statistically significant. Analysis of the data and plotting of the figures were done with the aid of computer software (SigmaStat and SigmaPlot, San Rafael, CA, USA; GraphPad PRISM™, San Diego, CA, USA) run on an IBM compatible computer and a Power Macintosh.

RESULTS

There were no significant differences in the clinical characteristics, including age, body weight, height, hemoglobin, hematocrit, and platelet count among all groups (all *P* > 0.05) (Table I). Table II shows the hemodynamic features of all

subject groups. There were no significant differences in pulse rate, systolic and diastolic blood pressures, echocardiographic fractional shortening, or ejection fraction in all groups (all $P > 0.05$).

In terms of pulmonary circulation (Table II), the systolic pulmonary arterial pressure in group C was significantly higher than those in group A and group B (both $P < 0.01$). Within group C, the systolic pulmonary arterial pressure increased when the severity of the clinical CHF increased, but there was no significant difference ($P = 0.12$). The pulmonary vascular markings of the chest radiographs were significantly increased in group C compared to group B (60.5% versus 17.3%, $P < 0.0001$). Within group C, there were more patients with increased pulmonary vascular markings as the CHF became more severe, but there was no significant difference, except when group CIII was compared with group CI ($P < 0.05$).

Figure 1 shows the plasma CGRP levels in each group. In group C, the CGRP concentrations were markedly elevated as compared with those in group A (15.8 ± 2.1 versus 7.0 ± 0.8 pg/mL, $P < 0.05$) and group B (15.8 ± 2.1 versus 8.6 ± 1.2 pg/mL, $P < 0.01$). Within group C, the levels of CGRP increased as the severity of CHF increased (CI, 10.3 ± 2.5; CII, 11.7 ± 2.0; CIII, 24.0 ± 4.1 pg/mL, $P < 0.01$). When group C was compared with the control group, there were no significant differences between CI and the control ($P = 0.24$), but CII and CIII were significantly different from the control ($P < 0.05$ and $P < 0.001$, respectively). In addition, CIII was significantly different from the control, CI, and CII groups ($P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively).
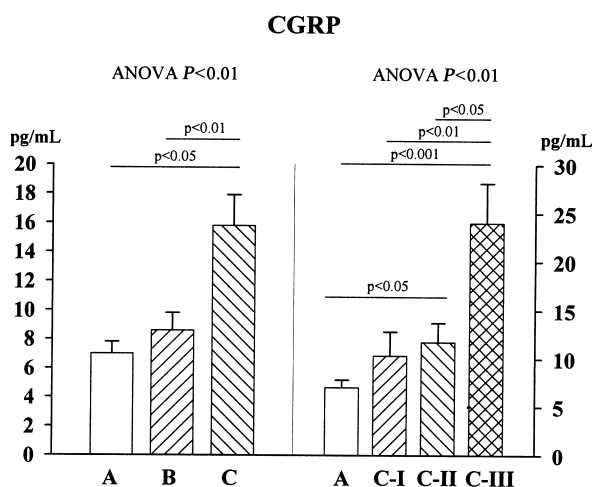

Figure 1. Comparison of plasma CGRP in normal subjects and patient groups with or without congestive heart failure.
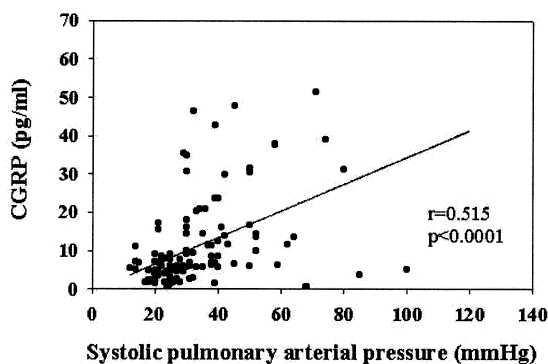
Figure 2. Relationship between the plasma concentrations of CGRP and systolic pulmonary arterial pressure in all patients.

After assessing the relationship between CGRP levels and systolic pulmonary arterial pressure in all of the subjects using linear regression (Figure 2), it was found that there was a significant positive correlation between plasma CGRP and systolic pulmonary arterial pressure (y = -0.56 + 0.35x, r = 0.515; $P < 0.0001$).

DISCUSSION

In adults, CHF can be caused by impaired systolic function and a range of neurohumoral substances have been suggested as markers for detection or evaluation of CHF.[16] However, in infants and children the most common etiology of CHF is CHD and increased pulmonary blood flow plays an important role in the pathogenesis in CHF secondary to CHD. A number of endogenous lung peptides are involved in maintaining balance in the pulmonary circulation, while endothelin-1 (ET-1) is the most potent vasoconstrictor and CGRP is the most important vasodilator with positive inotropic effects.[5-8] We have previously demonstrated the effects of increased pulmonary flow on ET-1 in animals with left to right shunt and in children with CHF caused by CHD.[17,18] The present study shows that plasma concentrations of CGRP are significantly elevated in relation to the clinical severity of CHF. Moreover, we have also demonstrated that increased pulmonary arterial pressure caused by left to right shunt correlates with increased plasma levels of CGRP. These results suggest that pulmonary overcirculation may be important for the regulation of CGRP production in pediatric CHF, which to our knowledge, has not been reported previously.

In the cardiovascular system, CGRP is mainly confined to the nerve fibers in the heart and around the blood vessels, and is involved in the regulation of a number of cardiovascular functions.[4] CGRP has positive inotropic and chronotropic effects on the heart and is an extremely potent central and peripheral vasodilator.[5-7] Unfortunately, previous reports on the changes in CGRP in CHF are conflicting. In adult patients, CHF is often caused by impaired systolic function, and the plasma concentration of CGRP here has been reported to be unaffected, decreased, or increased.[9-11] In other conditions with volume overload states, such as pregnancy, hepatic cirrhosis, or hemodialysis, increased levels of CGRP are also observed.[19-21] In hemodialysis patients, the plasma levels of CGRP were significantly higher in patients with fluid excess and correlated positively with the degree of fluid overload.[21] In our patients, the CHF was mainly due to pulmonary flow overload rather than systolic dysfunction, as many of our patients with CHF have normal echocardiographic ejection fractions but increased radiographic pulmonary vascular markings. Consistent with previous studies regarding fluid overload, our results revealed that plasma CGRP levels are also increased in states of volume overload in the pulmonary circulation. We have also demonstrated for the first time that CGRP may be a marker of CHF secondary to CHD with left to right shunt, especially when CHF is severe.

CGRP is believed to play an important role in the modulation of pulmonary vascular tone, but the regulation of CGRP production is not fully understood. CGRP effectively dilates precontracted pulmonary arteries *in vitro*.[22] Recently, it was found that in patients with systemic sclerosis and pulmonary hypertension, there is a positive correlation between CGRP and systolic pulmonary arterial pressure.[23] The similar positive correlation between CGRP and pulmonary pressure in our study suggests that changes in pulmonary arterial pressure may possibly play a role in the mechanism of CGRP production. Tjen, *et al* previously showed that exogenous CGRP reduced pulmonary artery pressure in hypobaric hypoxic rats.[24] However, in this animal model, the increase in pulmonary arterial pressure correlates with declining blood CGRP levels.[25] The possible explanation for the difference may lie in the fact that in CHD with left to right shunt, the pulmonary circulation is often hyperoxic rather than hypoxic. Accordingly, we propose that in patients with increased pulmonary arterial pressure caused by fluid overload, the mechanism of CGRP regulation may be different from that with hypoxic pulmonary hypertension.

Previous studies have demonstrated that CGRP has inhibitory effects on sympathetic nervous activity in the central nervous system.[26,27] The sympathetic nervous system plays a major role in cardiovascular adaptation to CHF, which is associated with augmented activity of the sympathetic nervous system, reflected in increased norepinephrine excretion. We have previously reported that elevated

plasma norepinephrine levels occur concurrently with increases in the severity of CHF in children with CHD.[28] In addition to the sympathetic system, CGRP is also closely related to ET-1. It was recently found in rats that CGRP plays a compensatory role in preventing ET-1-induced elevation in blood pressure.[29] In a rat model of CHF with pulmonary hypertension, we also showed that the endothelin system is upregulated.[30] In the present study, even though the exact regulatory mechanisms of CGRP production are unclear and require investigation in the future, we assume that the increase in CGRP in CHF may be considered as a compensatory response to counteract the increased pulmonary arterial pressure or vasoconstrictor effects of the sympathetic or endothelin system.

The limitation of this study concerns the relatively small number of patients assigned to each subgroup of CHF (group C) and the hemodynamic changes regarding pulmonary circulation in radiography or echocardiography among these subgroups were mostly not statistically significant, although they may well be clinically meaningful. A much larger population would have been required to confirm or refute this. Another limitation is that the degree of increased pulmonary flow could only be evaluated indirectly by the radiographic pulmonary vascular markings rather than directly measured because invasive cardiac catheterization was not justified in most of our patients. To minimize the bias in the assessment by chest radiography, the chest radiograph was interpreted by multiple observers in our study.

In conclusion, CHF secondary to CHD with increased pulmonary flow is associated with elevated levels of CGRP that are related to disease severity. Systolic pulmonary arterial pressure is positively correlated with levels of CGRP and pulmonary overcirculation may play a role in the upregulation of CGRP in CHF.

REFERENCES

1. Rosenfeld MG, Mermod JJ, Amara SG, *et al*. Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. Nature 1983; 304: 129-35.
2. Morris HR, Panico M, Etienne T, Tippins J, Girgis SI, MacIntyre I. Isolation and characterization of human calcitonin gene-related peptide. Nature 1984; 308: 746-8.
3. Mulderry PK, Ghatei MA, Rodrigo J, *et al*. Calcitonin gene-related peptide in cardiovascular tissues of the rat. Neuroscience 1985; 14: 947-54.
4. Uddman R, Edvinsson L, Ekblad E, Hakanson R, Sundler F. Calcitonin gene-related peptide (CGRP): perivascular distribution and vasodilatory effects. Regul Pept 1986; 15: 1-23.
5. Franco-Cereceda A, Bengtsson L, Lundberg JM. Inotropic effects of calcitonin gene-related peptide, vasoactive intestinal polypeptide and somatostatin on the human right atrium *in vitro*. Eur J Pharmacol 1987; 134: 69-76.
6. Brain SD, Williams TJ, Tippins JR, Morris HR, MacIntyre I. Calcitonin gene-related peptide is a potent vasodilator. Nature 1985; 313: 54-6.
7. Struthers AD, Brown MJ, Macdonald DW, *et al*. Human calcitonin gene-related peptide: a potent endogenous vasodilator in man. Clin Sci 1986; 70: 389-93.

8. Keith IM. The role of endogenous lung neuropeptides in regulation of the pulmonary circulation. Physiol Res 2000; 49: 519-37. (Review)

9. Valdemarsson S, Bergdahl A, Edvinsson L. Relationships between plasma levels of catecholamines and neuropeptides and the survival time in patients with congestive heart failure. J Intern Med 1994; 235: 595-601.

10. Taquet H, Komajda M, Grenier O, et al. Plasma calcitonin gene-related peptide decreases in chronic congestive heart failure. Eur Heart J 1992; 13: 1473-6.

11. Ferrari R, Panzali AF, Poole-Wilson PA, Anand IS. Plasma CGRP-like immunoreactivity in treated and untreated congestive heart failure. Lancet 1991; 338: 1084.

12. Ross RD, Bollinger RO, Pinsky WW. Grading the severity of congestive heart failure in infants. Pediatric Cardiol 1992; 13: 72-5.

13. Vazquez de Prada JA, Ruano J, Martin-Duran R, et al. Noninvasive determination of pulmonary arterial systolic pressure by continuous wave Doppler. Int J Cardiol 1987; 16: 177-84.

14. Marx GR, Allen HD, Goldberg SJ. Doppler echocardiographic estimation of systolic pulmonary artery pressure in pediatric patients with interventricular communications. J Am Coll Cardiol 1985; 6: 1132-7.

15. Parida SK, Schneider DB, Stoss TD, Pauly TH, McGillis JP. Elevated circulating calcitonin gene-related peptide in umbilical cord and infant blood associated with maternal and neonatal sepsis and shock. Pediatr Res 1998; 43: 276-82.

16. Groenning BA, Nilsson JC, Sondergaard L, Kjaer A, Larsson HB, Hildebrandt PR. Evaluation of impaired left ventricular ejection fraction and increased dimensions by multiple neurohumoral plasma concentrations. Eur J Heart Fail 2001; 3: 699-708.

17. Yeh JL, Hsu JH, Dai ZK, Liou SF, Chen IJ, Wu JR. Increased circulating big endothelin-1, endothelin-1 and atrial natriuretic peptide in infants and children with heart failure secondary to congenital heart disease. Int J Cardiol 2005; 104: 15-20.

18. Dai ZK, Tan MS, Chai CY, Chen IJ, Jeng AY, Wu JR. Effects of increased pulmonary flow on the expression of endothelial nitric oxide synthase and endothelin-1 in the rat. Clin Sci 2002; 103: Suppl 48: 289S-93.

19. Saggese G, Bertelloni S, Baroncelli GI, Pelletti A, Benedetti U. Evaluation of a peptide family encoded by the calcitonin gene in selected healthy pregnant women. A longitudinal study. Horm Res 1990; 34: 240-4.

20. Bendtsen F, Schifter S, Henriksen JH. Increased circulating calcitonin gene-related peptide (CGRP) in cirrhosis. J Hepatol 1991; 12: 118-23.

21. Odar-Cederlof I, Theodorsson E, Ericsson F, Kjellstrand CM. Plasma concentrations of calcitonin gene-related peptide in fluid overload. Lancet 1991; 338: 411-2.

22. Martling CR, Matran R, Alving K, Hokfelt T, Lundberg JM. Innervation of lower airways and neuropeptide effects on bronchial and vascular tone in the pig. Cell Tissue Res 1990; 260: 223-33.

23. Bartosik I, Eskilsson J, Ekman R, Akesson A, Scheja A. Correlation between plasma concentrations of calcitonin gene-related peptide and pulmonary pressure in patients with systemic sclerosis. Ann Rheum Dis 2002; 61: 261-3.

24. Tjen AL S, Ekman R, Lippton H, Cary J, Keith I. CGRP and somatostatin modulate chronic hypoxic pulmonary hypertension. Am J Physiol 1992; 263: H681-90.

25. Keith IM, Tjen AL S, Kraiczi H, Ekman R. Three-week neonatal hypoxia reduces blood CGRP and causes persistent pulmonary hypertension in rats. Am J Physiol Heart Circ Physiol 2000; 279: H1571-8.

26. Kawasaki H, Nuki C, Saito A, Takasaki K. Role of calcitonin gene-related peptide-containing nerves in the vascular adrenergic neurotransmission. J Pharmacol Exp Ther 1990; 252: 403-9.

27. Tsuda K, Tsuda S, Goldstein M, Masuyama Y. Effects of calcitonin gene-related peptide on ^{3}H norepinephrine release in medulla oblongata of spontaneously hypertensive rats. Eur J Pharmacol 1990; 191: 101-5.

28. Wu JR, Chang HR, Chen SS, Huang TY. Circulating noradrenaline and beta-adrenergic receptors in children with congestive heart failure. Acta Paediatr 1996; 85: 923-7.

29. Wang Y, Wang DH. Prevention of endothelin-1-induced increases in blood pressure: role of endogenous CGRP. Am J Physiol Heart Circ Physiol 2004; 287: H1868-74.

30. Dai ZK, Tan MS, Chai CY, et al. Upregulation of endothelial nitric oxide synthase and endothelin-1 in pulmonary hypertension secondary to heart failure in aorta-banded rats. Pediatr Pulmonol 2004; 37: 249-56.