Enhanced peripheral chemoreflex function in conscious rabbits with pacing-induced heart failure

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Sun, Shu-Yu, W. Wang, I. H. Zucker, and H. D. Schultz. Enhanced peripheral chemoreflex function in conscious rabbits with pacing-induced heart failure. *J. Appl. Physiol.* 86(4): 1264–1272, 1999.—The present study aimed to determine whether peripheral and/or central chemoreflex function is altered in chronic heart failure (CHF) and whether altered chemoreflex function contributes to sympathetic activation in CHF. A rabbit model of pacing-induced CHF was employed. The development of CHF (3–4 wk of pacing) was characterized by an enlarged heart, an attenuated contractility, and an elevated central venous pressure. Renal sympathetic nerve activity (RSNA) and minute volume (MV) of ventilation in response to stimulation of peripheral chemoreceptors by isocapnic/hypoxic gases were measured in the conscious state. It was found that the baseline RSNA at normoxia was higher in CHF rabbits than in sham rabbits (35.00 ± 4.03 vs. 20.75 ± 2.87% of maximum, $P < 0.05$). Moreover, the magnitudes of changes in RSNA and MV in response to stimulation of the peripheral chemoreceptors and the slopes of RSNA-arterial P_{O_2} and MV-arterial P_{O_2} curves were greater in CHF than in sham rabbits. Inhibition of the peripheral chemoreceptors by inhalation of 100% O_2 decreased RSNA in CHF but not in sham rabbits. The central chemoreflex function, as evaluated by the responses of RSNA and MV to hyperoxic/hypercapnic gases, was not different between sham and CHF rabbits. These data suggest that an enhancement of the peripheral chemoreflex occurs in the rabbit model of pacing-induced CHF and that the enhanced peripheral chemoreflex function contributes to the sympathetic activation in the CHF state.

renal sympathetic nerve activity; minute ventilation; hypoxia; hypercapnia

ONE OF THE MOST PROFOUND and reproducible changes that occur in chronic heart failure (CHF) is an augmentation of sympathetic nervous function. This has been repeatedly documented either in patients with CHF (9, 14) or in experimental models of CHF (11, 26). A popular hypothesis for the generalized sympathetic activation in CHF has been impairment of inhibitory arterial and cardiac baroreflexes (17, 27). However, other evidence suggests that excitatory reflexes may also play a role (2, 3, 16). Peripheral chemoreceptor activation is an excitatory input that results in increased sympathetic outflow and blood pressure (18). Recent studies have shown that the peripheral chemoreflex sensitivity is increased in patients with heart failure (2–5). It is not known whether enhanced chemoreflex sensitivity is also evident in experimental models of CHF.

Although the stimulation of peripheral chemoreceptors results in increased sympathetic outflow (18), it is still unclear whether the enhanced peripheral chemoreflex function contributes to the sympathetic activation in CHF. Clinical studies have documented an unchanged sympathetic tone in CHF patients (10, 24) when the afferent input from the peripheral chemoreceptors was inhibited by inhalation of 100% O_2. However, spectral analysis of heart rate (HR) variability has suggested that the peripheral chemoreflex tonically contributes to autonomic balance in CHF patients (20). Studies of chemoreflex function have not been conducted in experimental models of CHF.

A rabbit model of pacing-induced CHF has been established in this laboratory, which has been used for the investigation of baroreflex control of cardiovascular function and sympathetic nerve activity in CHF (15, 19). This rabbit model of CHF exhibits a similar neurohumoral activation and a blunted arterial baroreflex as those observed in patients with CHF (15, 19). The purpose of the present study was to evaluate whether peripheral and/or central chemoreflex function is altered in pacing-induced CHF and whether altered chemoreflexes contribute to the sympathetic activation. Renal sympathetic nerve activity (RSNA) and ventilation were measured in conscious state. Their responses to stimulation of peripheral and central chemoreceptors were examined to characterize the sensitivity of the peripheral and central chemoreflex, respectively.

MATERIALS AND METHODS

Thoracotomy

All experiments were carried out on male New Zealand White rabbits weighing 2.5–3.5 kg. Experiments were approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee and were carried out in accordance with the National Institutes of Health and the American Physiological Society's Guides for the Care and Use of Laboratory Animals. Rabbits were anesthetized with an anesthetic mixture consisting of 1.2 mg/kg acepromazine, 5.9 mg/kg xylazine, and 58.8 mg/kg ketamine, given as an intramuscular injection. Supplemental anesthesia was provided by intravenous pentobarbital sodium at a dose of 1.7 mg/kg as needed.

With the use of sterile technique, a left thoracotomy was performed as previously described (19). Briefly, the pericardium was opened, and a pin electrode of our own design was implanted on the left ventricle for pacing. Two sonomicrometer crystals (Sonometrics) were attached to the epicardial surfaces of the opposite walls at the base of the heart to measure external cardiac diameter. All leads exited the chest

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between the 3rd and 4th ribs. A ground lead was implanted on the left wall of the chest underneath the skin. The chest was then closed in layers and evacuated. Proper procedures were taken to ensure atelectasis and pneumothorax were minimized. Rabbits were placed on an antibiotic regimen consisting of 2.3 mg/kg im Baytril for 5 days.

Production of Heart Failure

After the rabbits recovered from the thoracotomy (10–14 days), baseline cardiac end-systolic and end-diastolic external diameters, fractional shortening, and shortening velocity (dD/dt_{max}) were measured by sonomicrometry. An arterial blood sample (0.25 ml) was taken by needle puncture of an ear artery for measuring blood gases and pH. After these prepacing measurements were completed, a pacing regimen was started. The pacing was started at 320 beats/min, which was held for 7 days, and then the rate was gradually increased to 380 beats/min, with an increment of 20 beats/min each week. The pacemaker was of our own design, with its output usually being set at 4–5 V and 0.5 ms. Sonograms and blood gases were performed weekly, with the rabbits sitting quietly in a Plexiglas box and with the pacemaker turned off for at least 60 min before the recordings were started. Rabbits with >40% reductions in dD/dt_{max} and shortening fraction were considered as being in CHF (generally taking 3–4 wk). Sham-operated rabbits underwent a similar period of sonographic measurements. No rabbits in the study exhibited abnormal arterial blood gases [either arterial P_{O_2} (Pa_{O_2}) <80 Torr, arterial P_{CO_2} (Pa_{CO_2}) >45 Torr, or Pa_{CO_2} <30 Torr] while breathing room air at rest during the course of the pacing regimen.

Implantation of Renal Nerve Electrodes and Catheters

In the paced and sham animals, 3–5 days before the experiment, a pair of electrodes was implanted on the left renal sympathetic nerves. A ground electrode was sutured to the perirenal fat. These procedures have been previously described in detail (19). The electrode wires were tunneled beneath the skin to exit on the upper back.

A MicroRenathane catheter was inserted into the right carotid artery for measurement of arterial blood pressure (ABP), and another catheter was inserted via the jugular vein into the superior vena cava for measurement of central venous pressure (CVP) and for administration of drugs. The catheters were tunneled beneath the skin and filled with heparin (1,000 U/ml). Rabbits were allowed to recover for 3–5 days, depending on the quality of RSNA, before experiments commenced.

Recording Techniques

ABP and CVP were recorded from the arterial and venous catheters, respectively, by using Hewlett-Packard transducers and Coulburn bridge amplifiers. Mean blood pressure (MBP) and HR were calculated from the pulsatile arterial pressure by using a software-analysis package (MacLab).

RSNA was recorded by using a Grass P511 differential amplifier and a storage oscilloscope. The RSNA was filtered at a bandwidth of 100 Hz-3 kHz. The neural signal was also fed to an audio amplifier and loudspeaker. The neural signal was rectified, integrated (1- or 5-s time constant), and both the raw and integrated signal were recorded by using the MacLab software. RSNA was corrected for noise by subtracting the integrated noise level from the total integrated signal. The noise was determined at the end of the experiment by bolus injection of phenylephrine to increase MBP to at least 130 mmHg to inhibit sympathetic outflow.

Tidal volume (TV) and breathing rate (BR) were determined by unrestrained plethysmography described previously (1, 12) and modified to our requirements. Rabbits were placed in a Plexiglas chamber (volume 11 liters) with exit ports for catheters and renal nerve electrodes. The chamber was sealed, except for an inlet and outlet port that allowed a continuous flow of air through the chamber. Different gas mixtures could be easily passed through the chamber to alter blood gases. TV was measured by temporarily (30 s) sealing the air ports and measuring the pressure changes in the sealed chamber by using a Validyne (MP-45) differential pressure transducer and amplifier connected to the MacLab analysis system. Chamber pressure fluctuations are proportional to TV because inspired gas expands in the lungs as it is warmed to body temperature and water vapor is added to it. The expansion of inspired gas causes compression of the air in the chamber. The chamber was sealed only for short intervals (30 s) to prevent appreciable changes in the composition of air in it. All test gases were saturated with water vapor by passage through a warmed bubbling chamber.

Calibration of TV was performed dynamically, with the animal present in the chamber by varying the volume of the chamber with a calibrated plunger at 60 cycles/min. TV was calculated from the formula of Drorbaugh and Fenn (7)

$$TV = V_{cal}P_mT_a(P_B - Pc_{H_2O})/$$

$$P_{cal}[T_a(P_B - Pc_{H_2O}) - T_c(P_B - Pa_{H_2O})]$$

where V_{cal} is calibration volume, P_m are pressure excursions due to breathing, P_{cal} are pressure excursions due to the calibration volume, and T_a is rectal temperature in °K. The actual rectal temperatures in sham and CHF rabbits were 39.2 ± 0.4 and 39.1 ± 0.2°C ($P > 0.05$), respectively; T_c is chamber air temperature in °K; P_B is barometric pressure (≈735 mmHg in this laboratory); Pa_{H_2O} is water vapor pressure at body temperature, assumed to be 52 mmHg; and Pc_{H_2O} is water vapor pressure at chamber air temperature. This was derived from T_c, assuming complete saturation of the chamber gas with water vapor. In several early experiments, the relative humidity in the chamber was measured and was always found to be 100%. BR was calculated from the excursion of TV signal by using a rate meter built into the MacLab system.

The P_{O_2} and P_{CO_2} and pH of arterial blood were measured by using a blood-gas analyzer (ABL5, Radiometer). Arterial blood samples were drawn from the arterial catheter during the steady state of the response to various stimuli.

Protocols

After complete recovery from surgery, rabbits were placed in the plethysmography chamber, and the catheter and the electrodes were connected for measurement of ABP, CVP, and RSNA, as explained above. The rabbits had been trained to sit quietly within the chamber throughout the experiment. The pacemaker was turned off for at least 60 min in paced rabbits before any recordings were taken. A control arterial blood gas was taken, and any metabolic acidosis was corrected by intravenous injection of an appropriate amount of $NaHCO_3$ (0.2 × body weight × base excess meq).

Peripheral chemoreflex control of RSNA and ventilation. Changes in RSNA, TV, and BR in response to stimulation of peripheral chemoreceptors were measured in eight sham and eight CHF rabbits in the conscious resting state. Peripheral chemoreceptors were stimulated preferentially by allowing the rabbits to breathe graded mixtures of hypoxic gas under isocapnic conditions. Different concentrations of O_2 with

balance of N_2 were delivered into the chamber in the following sequence: 21% O_2 (normoxia), 15% O_2 (mild hypoxia), and 10% O_2 (severe hypoxia). Each stimulation was held until a steady response was achieved (3–5 min). Then the RSNA, TV, BR, MBP, and HR were measured during the 30-s plethysmograph maneuver (explained above), and an arterial blood sample (0.5 ml) was taken from the arterial catheter for the measurement of Pa_{O_2}, Pa_{CO_2}, and pH. Because hypoxic stimulation of ventilation induces hyperventilatory hypocapnia, 2–4% CO_2 were added to the hypoxic gases to maintain relatively constant Pa_{CO_2} during hyperventilation. While the rabbits were breathing control air (21% O_2), sufficient recovery time was allowed between stimuli to ensure that all variables returned to baseline levels.

Central chemoreflex control of RSNA and ventilation. Changes in RSNA, TV, and BR in response to stimulation of central chemoreceptors were measured in eight sham and eight CHF rabbits in the conscious resting state. Central chemoreceptors were stimulated preferentially by allowing the rabbits to breathe hypercapnic gases during hyperoxia to inhibit peripheral chemoreceptor input. Test gases with graded levels of CO_2 were introduced into the chamber in the following sequence: 0% CO_2 (isocapnia), 7% CO_2 (mild hypercapnia), and 15% CO_2 (severe hypercapnia) in 50% O_2 and with balance of N_2. Each stimulation was held until a steady response was achieved (2–4 min). Then the RSNA, TV, BR, MBP, and HR were measured during the 30-s plethysmograph maneuver (explained above), and an arterial blood sample (0.5 ml) was taken from the arterial catheter for the measurement of Pa_{O_2}, Pa_{CO_2}, and pH.

Effect of inhibition of peripheral chemoreceptors on RSNA and ventilation. We found in the first protocol an elevated baseline RSNA in the normoxic state and an enhanced response of RSNA induced by hypoxia in CHF rabbits. The following protocol was conducted in seven sham and seven CHF rabbits to determine whether the elevated baseline RSNA in CHF rabbits was due to the enhanced peripheral chemoreceptor input at rest. After the baseline values of RSNA and ventilation were measured in the normoxic state, 100% O_2 was delivered into the chamber for 10 min to deactivate the peripheral chemoreceptors, and then the RSNA and ventilation were measured again with the application of 100% O_2 continued. An arterial blood sample was taken at the end of each period for the measurement of Pa_{O_2}, Pa_{CO_2}, and pH.

Contribution of pulmonary afferents to the augmentation of RSNA in response to hypoxia. An increased TV in response to the chemoreflex stimulation augments a secondary central input from pulmonary afferent endings, which is known to influence chemoreflex responses in humans (22). To assess a potential influence of pulmonary afferents on chemoreflex control of RSNA in our study, we conducted the following protocol in six normal rabbits. Rabbits were anesthetized with the same anesthetic mixtures used for surgical procedures (see above), so that we could mechanically control ventilation and thus pulmonary afferent input. The discharge level of RSNA in the normoxic state and its response to hypoxia were examined when the rabbit was allowed to breathe spontaneously and when the rabbit was ventilated at a constant TV and rate by a ventilator. The TV and BR during the spontaneous ventilation were measured by using a flowmeter (8300, Hans Rudolph). The same volume and rate as that at rest during spontaneous ventilation were applied during the mechanical ventilation. A short-acting neuromuscular blocker (pancuronium bromide, 0.25 mg/kg iv) was administered during mechanical ventilation to prevent breathing efforts. Isocapnic hypoxia was produced by applying gas mixtures of graded concentrations of O_2 to the inlet of the ventilator (mechanical ventilation) or to the opening of the tracheal tubing (spontaneous ventilation). During hypoxia-induced hyperventilation with spontaneous breathing, the gas mixtures were supplemented with CO_2 to maintain isocapnia.

Data Analysis

RSNA was expressed as the percent of the maximal nerve activity evoked by eliciting the oropharyngeal reflex with a puff of cigarette smoke as previously described (15), determined after completing the experiment. The respiratory

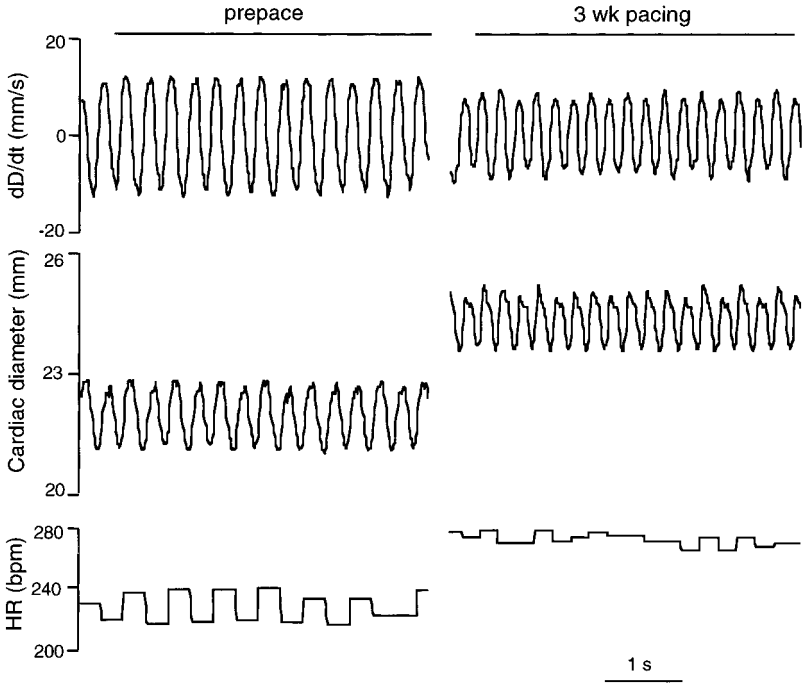

Fig. 1. Chart recordings of cardiac diameter (*D*), contractility (d*D*/d*t*), and heart rate (HR) from a rabbit before and after 3 wk of cardiac pacing. Rabbit exhibited an increased *D*, a decreased d*D*/d*t*, and an increased HR after 3 wk of pacing. bpm, Beats/min.

Table 1. *Baseline hemodynamics, heart weight, and blood-gas parameters in sham and CHF rabbits after chronic pacing*

	Sham ($n = 8$)	CHF ($n = 8$)
MBP, mmHg	80 ± 2	73 ± 3*
HR, beats/min	236 ± 11	289 ± 24*
CVP, mmHg	−0.9 ± 0.1	5.1 ± 0.7*
LVW, mg/kg	932 ± 14	1144 ± 43*
WHW, mg/kg	2410 ± 62	3325 ± 51*
Pa_{O_2}, Torr	93.3 ± 2.5	91.3 ± 2.9
Pa_{CO_2}, Torr	34.8 ± 1.8	35.8 ± 1.8
pH	7.438 ± 0.003	7.422 ± 0.010

Values are means ± SE; n = no. of rabbits. CHF, chronic heart failure; MBP, mean arterial blood pressure; HR, heart rate; CVP, central venous pressure; LVW, left ventricular wt/body wt; WHW, whole heart wt/body wt; Pa_{O_2}, arterial P_{O_2}; Pa_{CO_2}, arterial P_{CO_2}. *$P <$ 0.05, CHF vs. sham.

response was described by minute volume of ventilation ($\dot{V}_I$). $\dot{V}_I$ was calculated from TV and BR ($\dot{V}_I$ = TV × BR) and was normalized to body weight.

Peripheral chemoreflex function curves were analyzed by plotting data points averaged over 30 s for RSNA and $\dot{V}_I$ against the corresponding Pa_{O_2}. A three-parameter hyperbolic equation was fitted to the data as described previously (13). The equation is described as RSNA or $\dot{V}_I = a + b/(Pa_{O_2} - c)$. The values a, b, and c are constants, with a being the horizontal asymptote, b a shaping coefficient, and c the vertical asymptote. These parameters derived from the equation allowed us to quantify and compare the peripheral chemoreflex characteristics between sham and CHF rabbits. The values of r^2 from each curve fitting were from 0.96 to 0.99 ($P < 0.01$).

Central chemoreflex function was analyzed by plotting data points averaged over 30 s for RSNA and $\dot{V}_I$ against the corresponding Pa_{CO_2}. A linear regression was used for the analysis of these data. The equation is described as RSNA or $\dot{V}_I = A + B \times Pa_{CO_2}$, where A is the horizontal interception and B is the slope. These parameters allowed us to quantify and compare the central chemoreflex characteristics between sham and CHF rabbits. The values of r^2 from each linear regression were from 0.98 to 1.0 ($P < 0.01$).

The changes of RSNA and $\dot{V}_I$ in response to graded levels of Pa_{O_2} or Pa_{CO_2} in sham and CHF rabbits were analyzed by using a two-way ANOVA for repeated measures. A Bonferroni procedure was used as the post hoc test to determine the significance among treatments or between groups. The parameters derived from the curve fittings for peripheral and central chemoreflex function and the baseline hemodynamics were compared between sham and CHF rabbits by using an unpaired Student's t-test. Statistical analyses and curve fitting were performed by using commercial statistical software (GB-STAT, Dynamic Microsystems). All data are means ± SE. Statistical significance was accepted when $P < 0.05$.

RESULTS

Characteristics of the CHF State

Rapid ventricular pacing, as described in MATERIALS AND METHODS, induced CHF by the 3rd or 4th week of pacing. The CHF was characterized by an enlarged heart evaluated by the changes in cardiac dimensions and heart weight (Fig. 1, Table 1), an attenuated contractility was evaluated by the dD/dt_{max} and the fraction shortening (Fig. 1, Table 2) and an elevated CVP (Table 1). The paced rabbits also exhibited a slight decrease in MBP and a significant increase in HR (Table 1). There was no appreciable difference between sham and CHF rabbits in blood-gas parameters at rest (Table 1).

Characteristics of the Peripheral Chemoreflex in Sham and CHF Rabbits

It was observed that the baseline RSNA (expressed as %maximum) in the resting, normoxic state was elevated in CHF rabbits (Figs. 2 and 3). Also enhanced was the response of RSNA to graded levels of isocapnic hypoxia in CHF rabbits (Fig. 3). The enhanced peripheral chemoreflex control of RSNA in CHF rabbits was characterized by a greater shaping coefficient b and a left shift in the vertical asymptote c, derived from the hyperbolic curve fitting for RSNA-Pa_{O_2} (Table 3). $\dot{V}_I$ at rest (normoxia) was not different between sham and CHF rabbits, but the hypoxia-induced ventilatory response was enhanced in CHF rabbits (Fig. 3, Table 3). TV was not different between sham and CHF rabbits either at rest or in response to equivalent levels of hypoxia (Table 4). BR at rest was not different between sham and CHF rabbits, but its response to isocapnic hypoxia was greater in CHF rabbits (Table 4).

Table 4 also summarizes MBP, HR, Pa_{CO_2}, and pH during isocapnic hypoxia in both groups. There were no significant changes in these variables during hypoxic stimulation.

Table 2. *Cardiac diameters and contractility in sham and CHF groups before and during the cardiac pacing regimen*

	Baseline	1 wk	2 wk	3–4 wk
Sham ($n = 8$)				
ESD, % of control	100.0 ± 0.0	99.6 ± 1.4	99.4 ± 1.1	100.5 ± 1.1
EDD, % of control	100.0 ± 0.0	99.5 ± 1.2	99.1 ± 1.0	100.0 ± 1.0
dD/dt_{max}, mm/s	−11.3 ± 1.5	−10.9 ± 1.1	−11.1 ± 1.2	−10.9 ± 1.2
%shortening	10.0 ± 0.7	10.0 ± 0.6	10.1 ± 0.6	10.0 ± 0.6
CHF ($n = 8$)				
ESD, % of control	100.0 ± 0.0	102.50 ± 1.1	112.50 ± 2.3*	114.7 ± 3.3*
EDD, % of control	100.0 ± 0.0	101.70 ± 0.7	108.20 ± 1.4*	109.9 ± 2.0*
dD/dt_{max}, mm/s	−10.3 ± 1.2	−7.3 ± 0.8*	−5.3 ± 0.4*	−4.9 ± 0.3*
%shortening	9.1 ± 0.6	6.4 ± 0.4*	5.2 ± 0.5*	4.5 ± 0.6*

Values are means ± SE; n = no. of rabbits. ESD, end-systolic diameter; EDD, end-diastolic diameter; dD/dt_{max}, 1st derivative of change in diameter; %shortening = (EDD − ESD)/EDD × 100%. *$P < 0.05$, compared with baseline.

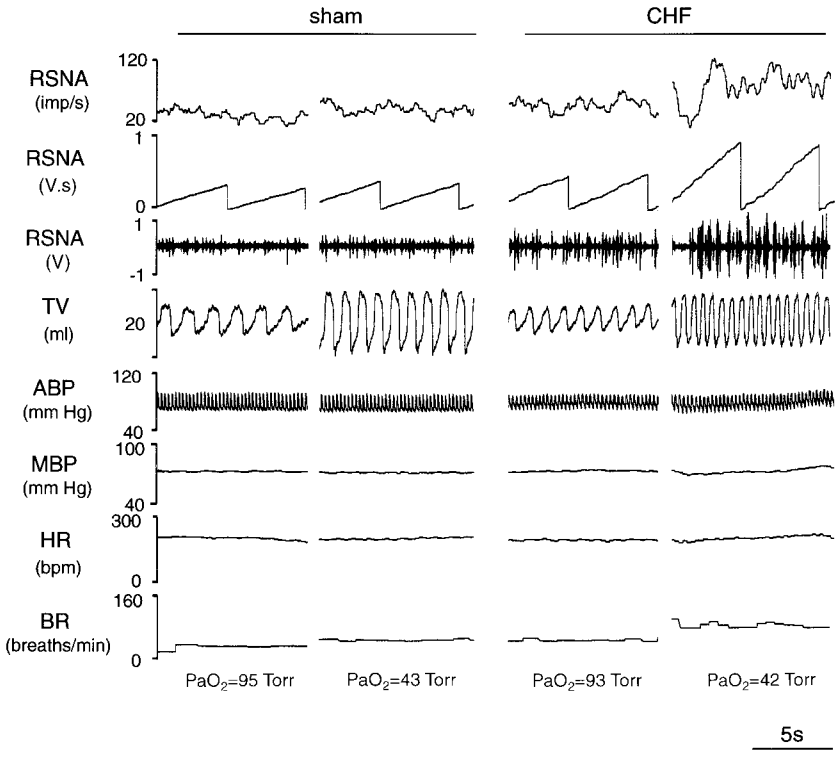

Fig. 2. Chart recordings of renal sympathetic nerve activity (RSNA), tidal volume (TV), arterial blood pressure (ABP), mean arterial blood pressure (MBP), HR, and breathing rate (BR) under normoxic and hypoxic conditions from a sham and a chronic hear failure (CHF) rabbit. CHF rabbit exhibited a higher level of baseline RSNA in normoxic state and an enhanced response to hypoxia in both RSNA and ventilation. Pa_{O_2}, arterial P_{O_2}.

Characteristics of the Central Chemoreflex in Sham and CHF Rabbits

The RSNA levels were higher in CHF rabbits than in sham rabbits at the equivalent levels of Pa_{CO_2} (Fig. 4). This upward shift of RSNA-Pa_{CO_2} relationship in CHF rabbits resulted from the higher baseline RSNA instead of from an enhanced sensitivity of the central chemoreflex, since the slopes of the RSNA-Pa_{CO_2} curves were not different between sham and CHF rabbits (Table 5). The hypercapnia-induced response in $\dot{V}_I$ was not different between sham and CHF rabbits (Fig. 4, Table 5). The changes in TV and BR in response to hypercapnia also were not different between sham and CHF rabbits (Table 6).

Table 6 also summarizes the blood-gas data during hyperoxic hypercapnia. There was an acidosis during the hypercapnic stimulation, as indicated by the decreases in pH. However, the extent of decrease in pH was not different between sham and CHF rabbits.

Effect of Inhibition of Peripheral Chemoreceptors on RSNA in Sham and CHF Rabbits

When the afferent input from peripheral chemoreceptors was inhibited by inhalation of 100% O_2, the discharge level of RSNA was attenuated in CHF rabbits but not in sham rabbits (Fig. 5). However, RSNA in CHF rabbits in the hyperoxic state was still higher, compared with that in sham rabbits (Fig. 5). Pa_{O_2} was not different between sham and CHF rabbits either in the normoxic state (93.0 + 2.5 vs. 91.3 ± 2.9 Torr, $P > 0.05$) or in the hyperoxic state (413.0 ± 9.3 vs. 400.0 ± 6.1 Torr, $P > 0.05$).

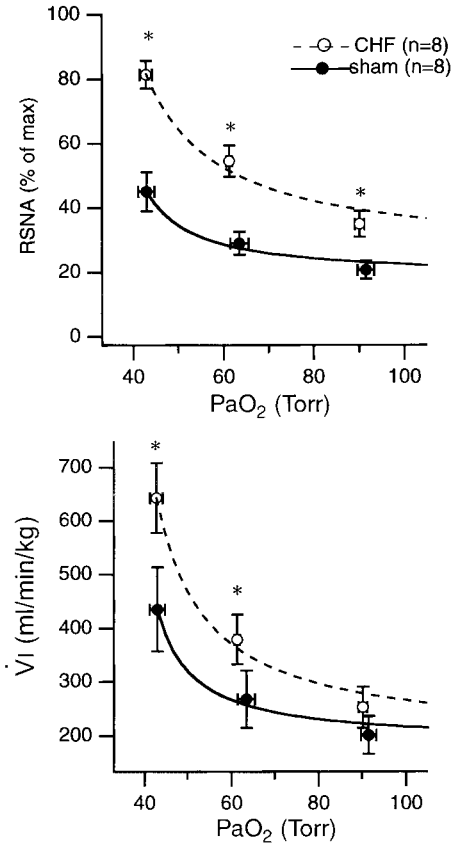

Fig. 3. RSNA-Pa_{O_2} (*top*) and minute ventilation ($\dot{V}_I$)-Pa_{O_2} relationships (*bottom*) in sham and CHF rabbits plotted by mean values of actual data (circles with error bars) and by fitted hyperbolic curves (lines). CHF rabbits exhibited a higher level of baseline RSNA in normoxic state (Pa_{O_2} at 90–95 Torr) and an enhanced response of RSNA and $\dot{V}_I$ to hypoxia. *$P < 0.05$, CHF vs. sham.

Table 3. *Logistic parameters derived from hyperbolic curve fitting for RSNA-Pa_{O_2} and $\dot{V}_I$-Pa_{O_2} relationships in sham and CHF rabbits*

	Sham ($n=8$)	CHF ($n=8$)
RSNA-Pa_{O_2}		
a, %maximum	16.6 ± 2.3	26.7 ± 2.5†
b, %maximum Torr	294.0 ± 87.6	930.5 ± 144.8†
c, Torr	33.2 ± 2.5	25.3 ± 2.3†
$\dot{V}_I$-Pa_{O_2}		
a, ml·min^{-1}·kg^{-1}	185.9 ± 20.1	191.6 ± 26.9
b, ml·min^{-1}·kg^{-1}· Torr	2,036.1 ± 456.9	5,122.1 ± 832.8†
c, Torr	34.9 ± 2.6	31.5 ± 3.6

Values are means ± SE; *n* = no. of rabbits. RSNA, renal sympathetic nerve activity; $\dot{V}_I$, minute ventilation; *a–c*, logistic parameters. See text for further explanation. *$P < 0.05$, CHF vs. sham.

MBP, HR, and $\dot{V}_I$ were not affected by the inhibition of peripheral chemoreceptors either in sham or in CHF rabbits (Table 7).

Effect of Ventilation Control on the RSNA Response to Hypoxia

TV and BR during normoxic, mild hypoxic, and severe hypoxic states while the rabbits were breathing either spontaneously or by controlled mechanical ventilation are summarized in Table 8. During controlled ventilation, the TV and rate for the ventilator were set according to the values measured from each rabbit in the normoxic state while it was breathing spontaneously. It was observed that an equivalent grade of hypoxia induced similar responses in RSNA with spontaneous respiration vs. constant TV and rate, as evaluated either by the magnitude of the hypoxia-induced response in RSNA (Fig. 6) or by the shaping coefficients from the hyperbolic curve fitting for RSNA-Pa_{O_2} (mechanical ventilation: 739.7 ± 92.4 %maximum·Torr of Pa_{O_2}; spontaneous breathing: 996.6 ± 153.7 %maximum·Torr of Pa_{O_2}, $P > 0.05$).

Table 4. *Hemodynamic, ventilatory, and blood-gas variables during isocapnic hypoxia in sham (n = 8) and CHF (n = 8) rabbits*

	Normoxia	Mild Hypoxia	Severe Hypoxia
MBP, mmHg			
Sham	80 ± 3	77 ± 3	79 ± 2
CHF	73 ± 3‡	71 ± 3‡	73 ± 4‡
HR, beats/min			
Sham	236 ± 12	238 ± 11	241 ± 9
CHF	289 ± 7‡	288 ± 9‡	291 ± 10‡
TV, ml			
Sham	10.9 ± 0.5	13.1 ± 1.2*	17.9 ± 2.2†
CHF	10.6 ± 1.5	13.6 ± 1.3*	17.1 ± 1.2†
BR, breaths/min			
Sham	60 ± 6	64 ± 6	77 ± 7*
CHF	66 ± 6	88 ± 9*‡	112 ± 10*‡
Pa_{O_2}, Torr			
Sham	91.5 ± 1.8	63.6 ± 2.0†	43.0 ± 1.8†
CHF	90.2 ± 1.0	61.4 ± 0.7†	43.8 ± 1.4†
Pa_{CO_2}, Torr			
Sham	34.8 ± 1.8	33.1 ± 1.3	32.1 ± 1.1
CHF	35.8 ± 1.8	33.5 ± 0.7	33.0 ± 1.7
pH			
Sham	7.438 ± 0.003	7.431 ± 0.004	7.428 ± 0.006
CHF	7.422 ± 0.010	7.420 ± 0.007	7.412 ± 0.013

Values are means ± SE. TV, tidal volume; BR, breathing rate. *$P <$ 0.05, †$P < 0.01$, vs. normoxia; ‡$P < 0.05$, CHF vs. sham.

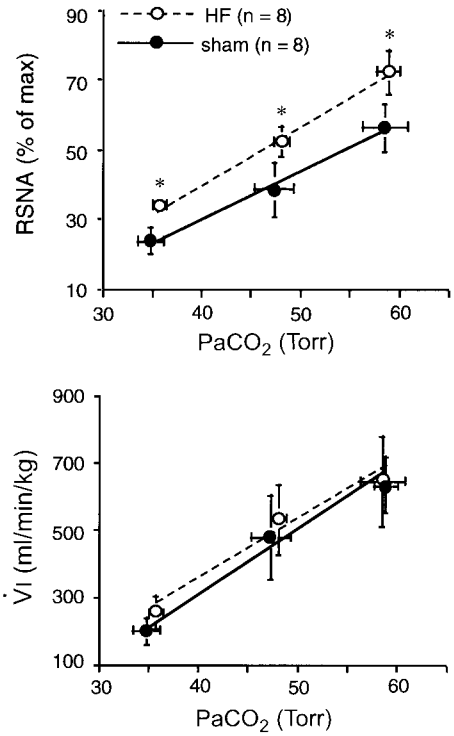

Fig. 4. RSNA- (*top*) and $\dot{V}_I$-arterial P_{CO_2} (Pa_{CO_2}) (*bottom*) relationships in sham and CHF rabbits plotted by mean values of actual data (circles with error bars) and by linear regressions (lines). CHF rabbits exhibited a higher level of RSNA in corresponding hypercapnic states, but slopes of linear regressions for RSNA- and $\dot{V}_I$-Pa_{CO_2} relationships were not different between sham and CHF rabbits. *$P < 0.05$, CHF vs. sham.

MBP was increased in response to hypoxia to a similar magnitude in both conditions. HR was not affected by hypoxia. Blood gases were not different between spontaneous breathing and constant TV under corresponding conditions (Table 8).

DISCUSSION

The principal findings of this study were as follows: *1*) the baseline RSNA at normoxia and the isocapnic, hypoxia-induced responses in RSNA and $\dot{V}_I$ were enhanced in CHF rabbits compared with sham rabbits; *2*) there was no significant difference in the hyperoxic,

Table 5. *Logistic parameters derived from linear regression for RSNA-Pa_{CO_2} and $\dot{V}_I$-Pa_{CO_2} relationships in sham and CHF rabbits*

	Sham ($n=8$)	CHF ($n=8$)
RSNA-Pa_{CO_2}		
A, %maximum	−31.5 ± 5.7	−30.4 ± 5.1
B, %/Torr	1.5 ± 0.2	1.8 ± 0.3
$\dot{V}_I$-Pa_{CO_2}		
A, ml·min^{-1}·kg^{-1}	−528.8 ± 107.5	−372.6 ± 97.8
B, ml·min^{-1}·kg^{-1} Torr^{-1}	20.4 ± 4.1	17.5 ± 3.8

Values are means ± SE; *n* = no. of rabbits. *A* and *B*, logistic parameters.

Table 6. *Hemodynamic, ventilatory, and blood-gas variables during hyperoxic hypercapnia in sham (n = 8) and CHF (n = 8) rabbits*

	Isocapnia	Mild Hypercapnia	Severe Hypercapnia
MBP, mmHg			
Sham	78 ± 3	83 ± 3	84 ± 3
CHF	73 ± 1.9‡	77 ± 3‡	74 ± 3‡
HR, beats/min			
Sham	234 ± 9	245 ± 12	243 ± 11
CHF	281 ± 7‡	285 ± 13‡	284 ± 15‡
TV, ml			
Sham	9.8 ± 1.2	16.1 ± 1.2*	23.5 ± 4.4*
CHF	10.6 ± 1.5	16.2 ± 1.2*	19.6 ± 1.0*
BR, breaths/min			
Sham	66 ± 6	78 ± 7*	92 ± 6*
CHF	67 ± 10	92 ± 10*	102 ± 10*
Pa_{CO_2}, Torr			
Sham	34.6 ± 0.6	46.9 ± 3.5†	58.7 ± 1.1†
CHF	35.6 ± 0.4	48.8 ± 0.6†	59.8 ± 1.0†
Pa_{O_2}, Torr			
Sham	241.5 ± 8.0	257.5 ± 8.4	259.6 ± 10.4
CHF	238.8 ± 9.0	250.7 ± 9.1	256.3 ± 10.2
pH			
Sham	7.438 ± 0.003	7.342 ± 0.008†	7.289 ± 0.009†
CHF	7.422 ± 0.010	7.346 ± 0.009†	7.295 ± 0.010†

Values are means ± SE. *$P < 0.05$, †$P < 0.01$ vs. isocapnia; ‡$P > 0.05$, CHF vs. sham.

hypercapnia-induced responses in RSNA and $\dot{V}_I$ between sham and CHF rabbits; *3*) inhibition of peripheral chemoreceptors by inhalation of 100% O_2 attenuated RSNA in CHF rabbits but not in sham rabbits. From these results, we conclude that an enhancement of the peripheral chemoreflex occurs in the rabbit model of pacing-induced CHF and that the enhanced peripheral chemoreflex function contributes to the sympathetic activation in the CHF state.

Sympathohumoral activation has been recognized as a key factor that influences the progression of the pathophysiological process and the prognosis of patients with CHF (8, 17, 26). However, the mechanisms for this alteration are not clear. A popular hypothesis

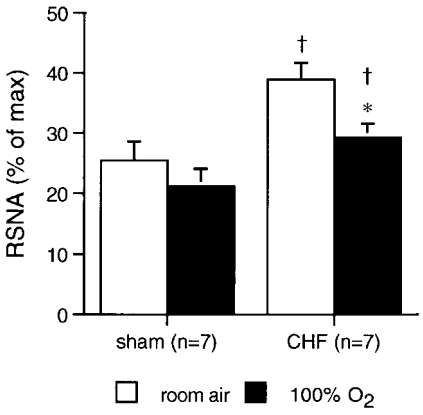

Fig. 5. Effect of inhibition of peripheral chemoreceptors by inhalation of 100% O_2 on RSNA in sham and CHF rabbits. Inhibition of peripheral chemoreceptors attenuated RSNA in CHF but not in sham rabbits. Inhalation of 100% O_2 elevated arterial P_{O_2} from 93.0 ± 2.5 to 413 ± 9.34 Torr in sham rabbits and from 91.3 ± 2.9 to 400 ± 6.1 Torr in CHF rabbits (not significant, sham vs. CHF). *$P < 0.05$, 100% O_2 vs. room air. †$P < 0.05$, CHF vs. sham.

Table 7. *Hemodynamics and minute ventilation in the normoxic and hyperoxic states in sham (n = 7) and CHF (n = 7) rabbits*

	Normoxia	Hyperoxia
MBP, mmHg		
Sham	78 ± 2	77 ± 3
CHF	70 ± 2†	69 ± 2*
HR, beats/min		
Sham	240 ± 13	238 ± 12
CHF	287 ± 10*	285 ± 9*
$\dot{V}_I$, $ml \cdot min^{-1} \cdot kg^{-1}$		
Sham	255 ± 60	250 ± 58
CHF	290 ± 39	287 ± 41

Values are means ± SE. *$P > 0.05$, CHF vs. sham.

for the sympathohumoral activation has been an impairment of the inhibitory arterial baroreflex (17, 27). However, recent studies have shown that chemoreflex sensitivity is augmented in patients with CHF (2–5), which may contribute to the sympathohumoral activation. The findings of the present study in the rabbit model of pacing-induced CHF confirm the previous observations in patients with CHF. An augmented

Table 8. *Hemodynamic, ventilatory, and blood-gas variables during isocapnic hypoxia in conditions of spontaneous and mechanical ventilation in anesthetized sham rabbits (n = 6)*

	Normoxia	Mild Hypoxia	Severe Hypoxia
MBP, mmHg			
Spontaneous ventilation	67 ± 2	73 ± 2*	81 ± 3*
Mechanical ventilation	70 ± 1	74 ± 2	83 ± 3*
HR, beats/min			
Spontaneous ventilation	289 ± 10	286 ± 11	288 ± 13
Mechanical ventilation	287 ± 9	285 ± 10	286 ± 12
TV, ml			
Spontaneous ventilation	15.4 ± 1.7	18.3 ± 2.2	24.0 ± 2.7*
Mechanical ventilation	15.4 ± 1.7	15.4 ± 1.7	15.4 ± 1.7
BR, breaths/min			
Spontaneous ventilation	47 ± 4	51 ± 6	59 ± 6*
Mechanical ventilation	47 ± 4	47 ± 4	47 ± 4
Pa_{O_2}, Torr			
Spontaneous ventilation	91.3 ± 1.6	60.5 ± 1.0†	41.1 ± 1.8†
Mechanical ventilation	93.0 ± 1.0	59.4 ± 0.7†	39.7 ± 1.4†
Pa_{CO_2}, Torr			
Spontaneous ventilation	36.8 ± 1.8	37.1 ± 1.7	36.9 ± 2.1
Mechanical ventilation	37.8 ± 1.8	38.5 ± 1.7	38.0 ± 2.0
pH			
Spontaneous ventilation	7.368 ± 0.005	7.351 ± 0.006	7.381 ± 0.003
Mechanical ventilation	7.352 ± 0.009	7.350 ± 0.005	7.350 ± 0.013

Values are means ± SE. *$P < 0.05$, †$P < 0.01$ vs normoxia.

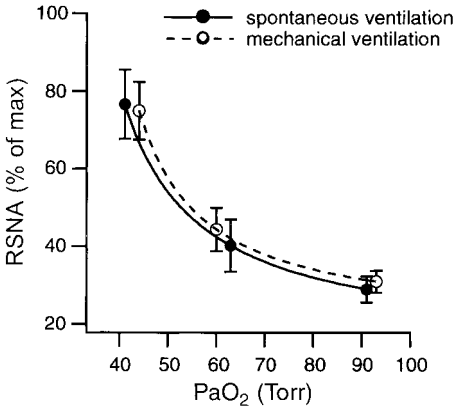

Fig. 6. Responses of RSNA to hypoxia in spontaneously and mechanically ventilated conditions in anesthetized rabbits. Magnitudes of RSNA in response to hypoxia were not different between the 2 conditions.

peripheral chemoreflex function in the CHF state was manifested in the present study by the increased magnitudes of RSNA and $\dot{V}_I$ in response to graded levels of hypoxia and by the larger shaping coefficients for RSNA- and $\dot{V}_I$-Pa_{O_2} curves in CHF rabbits. Because the Pa_{CO_2} and pH were controlled within the baseline levels during the hypoxic stimuli, the hypoxia-induced responses in RSNA and $\dot{V}_I$ should be solely mediated by activation of the peripheral chemoreceptors.

The finding that inhibition of peripheral chemoreceptors attenuated the discharge level of RSNA in CHF rabbits, but not in sham rabbits, indicates that the enhanced peripheral chemoreflex function contributes to sympathetic activation at rest in this rabbit model of CHF. This extends our knowledge of various reflex mechanisms that contribute to neurohumoral activation in the CHF state. A recent study in patients with CHF (20) found that there was a good correlation between an increased peripheral chemosensitivity and autonomic imbalance as reflected by spectral analysis of HR variability. Transient hyperoxia increased HR variability (an index of a decreased sympathetic activity and/or an increased parasympathetic activity) in those patients. This correlation agrees with our observation of a decreased RSNA during hyperoxia in the rabbit model of CHF. However, other studies (10, 24) found differing results. These studies reported that deactivation of peripheral chemoreceptors by breathing 100% O_2 did not affect resting muscle sympathetic nerve activity in patients with CHF. It is not clear why the results are inconsistent across studies. It is possible that these divergent results may reflect differences in chemoreflex control of sympathetic outflow to various vascular beds.

It should be noted that, even though RSNA was attenuated by 100% O_2 in CHF rabbits, the attenuated discharge level of RSNA after chemoreceptor inhibition was still higher in the CHF rabbits compared with the sham rabbits. This finding suggests that other factors, in addition to the enhanced peripheral chemoreflex, contribute to the sympathetic activation in the CHF state. These factors may include an impairment of inhibitory arterial and cardiac baroreflexes (17, 27) and/or an enhancement of an excitatory cardiac sympathetic reflex (16).

Peripheral chemoreflex control of ventilation was also enhanced in CHF rabbits as manifested by the increased change in $\dot{V}_I$ in response to hypoxia and by the larger shaping coefficient of the Pa_{O_2}-$\dot{V}_I$ curve. This enhanced ventilatory response in CHF rabbits resulted largely from an increased BR rather than TV, since the response in TV to hypoxia was not different between sham and CHF rabbits. The enhanced peripheral chemoreflex control of ventilation suggests a possible mechanism for the exaggerated exercise hyperpnea in patients with CHF.

An enhanced central chemoreflex function has been documented in patients with CHF, as manifested by a larger slope that related $\dot{V}_I$ to end-tidal pressure of CO_2 (3). However, by preferentially stimulating the central chemoreceptors with graded mixtures of hyperoxic/hypercapnic gases, we found no differences between sham and CHF rabbits in the central chemoreflex control of RSNA and $\dot{V}_I$, as evaluated by the slopes of the linear regressions for RSNA- and $\dot{V}_I$-Pa_{CO_2} relationships. The explanation for this discrepancy may be the difference in the species across studies or the duration and extent of heart failure.

Because the ventilatory response to hypoxia was enhanced in CHF rabbits, one must question whether secondary input from pulmonary afferents may have influenced differences in renal sympathetic responses between the sham and CHF groups. It is unlikely that the enhanced RSNA response to peripheral chemoreflex stimulation in the CHF rabbits was influenced by pulmonary afferents, since pulmonary afferents are known to inhibit, rather than enhance, chemoreflex control of sympathetic outflow (6, 22, 25). Moreover, we conducted experiments to define whether ventilatory status influences hypoxia-induced responses in RSNA in rabbits. It was observed that the ventilatory response to hypoxia did not affect the response in RSNA in the anesthetized rabbit. The question whether CHF itself alters pulmonary afferent input could not be addressed in the present study. Further studies are needed to assess whether pacing-induced heart failure alters pulmonary afferent function.

The important issue that arises from this study is defining the mechanism(s) responsible for this altered chemoreflex function. A companion study from our laboratory (23), in which we used the same rabbit model of pacing-induced CHF, has shown an enhanced afferent input from carotid body chemoreceptors in the CHF state, which would provide a primary contribution to the augmentation of reflex function. Another possible consideration is that baroreceptor input is known to have an important central interaction with peripheral chemoreflex responses to hypoxia (21). Studies have demonstrated a marked inverse relationship between baroreflex sensitivity and chemoreceptor drive in both normal subjects (21) and CHF patients (20). Thus impaired baroreflex function that is characteristic of heart failure (17, 26, 27) may contribute to the enhance-

ment of chemoreflex function. On the other hand, suppression of peripheral chemoreceptor drive by transient hyperoxia in CHF patients improves baroreflex sensitivity (20). Consequently, the extent of cause and effect between alterations in baroreflex and chemoreflex function as a result of their central interaction in heart failure remains to be resolved.

Significance

The findings from the present study in the rabbit model of CHF and those from previous studies in patients with CHF support the hypothesis that peripheral chemoreflex function is enhanced in CHF and that the enhanced peripheral chemosensitivity contributes to the sympathetic activation in the CHF state. This may shed light on the clinical treatment of CHF.

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