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Autonomic cardiovascular responses to hypercapnia in conscious rats: the roles of the chemo- and baroreceptors

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Abstract

The role of the autonomic nervous system, the central and peripheral chemoreceptors, and the arterial baroreceptors was examined in the cardiovascular response to hypercapnia in conscious rats chronically instrumented for the measurement of arterial blood pressure (ABP), heart rate (HR), and renal sympathetic nerve activity (RSNA). Rats were exposed to hypercapnia (6% CO_2), and the cardiovascular and autonomic nervous responses in intact and carotid chemo- and/or aortic denervated rats were compared. In intact and carotid chemo-denervated rats, hypercapnia induced significant increases in mean ABP (MABP) and RSNA, and a significant decrease in HR. The HR decrease was reversed by atropine and eliminated by bilateral aortic denervation, which procedure, however, did not affect the MABP or RSNA response. Bilateral carotid chemo-denervation did not affect the baroreflex control of HR, although this control was attenuated by aortic denervation. Hypercapnia did not affect baroreflex sensitivity in intact rats. These results suggest that hypercapnia induces an increase in MABP due to an activation of sympathetic nervous system via central chemoreceptors and a decrease in HR due to a secondary reflex activation of the parasympathetic nervous system via arterial baroreceptors in response to the rise in ABP. In addition, carotid chemoreceptors do not play a major role in the overall cardiovascular response to hypercapnia in conscious rats. The mechanism responsible for the parasympatho-excitation may also involve CO_2 induced aortic chemoreceptor simulation.

Keywords: Hypercapnia; Cardiovascular responses; Autonomic nervous system; Peripheral chemoreceptors; Arterial baroreceptors

1. Introduction

Carbon dioxide (CO_2) exerts significant effects on cardiovascular and respiratory systems mediated by central and/or peripheral chemoreceptors. Many authors have reported that hypercapnia elicits an increase in arterial blood pressure (ABP) due to sympatho-excitation (Richardson et al., 1961; Kollai and Koizumi, 1979; Rose et al., 1983; Fukuda et al., 1987; Somers et al., 1989). However, there have been a wide variety of results regarding the heart rate (HR) response, and various hypotheses have been proposed to explain the differences in these experimental results. Richardson et al. (1961) reported that hypercapnia induces an increase in HR through activation of the sympathetic nervous system. Fukuda et al. (1989) have suggested that an observed decrease in HR was due to a direct effect of CO_2 on cardiac pacemaker cells, but not due to the baroreflex response. In contrast, Walker and Brizzee (1990) have suggested that the baroreflex response does play an important role in the bradycardic response to hypercapnia. Other investigators have ascribed the HR responses to a reflex effect produced by stimulation of carotid or aortic chemoreceptors, but the results are also conflicting: stimulation of carotid chemoreceptor can evoke

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tachycardia (Karim et al., 1980) or bradycardia (Daly and Scott, 1958), stimulation of aortic chemoreceptor evoked tachycardia (Karim et al., 1980) or bradycardia (Angell James and Daly, 1969), when the carotid or aortic chemoreceptors were stimulated by hypercapnic blood or venous blood. The variability in regard to the role of autonomic nervous system in the HR responses or the effect of the chemoreceptor stimulation on HR might reflect a paucity of studies employing direct sympathetic nerve recordings in conscious animals or the difference in anesthetics used, since anesthesia is known to interfere with autonomic responses (Kannan et al., 1989; Shimokawa et al., 1998).

The present study was undertaken to determine the role of the autonomic nervous system in the regulation of the cardiovascular system during systemic hypercapnia by assessing sympathetic nerve activity in intact and atropine-treated conscious rats. Second, the effect of systemic hypercapnia have been examined in rats, with selective destruction of the carotid bodies, transection of the aortic nerves, both destruction of carotid bodies and transection of the aortic nerves, or sinoaortic denervation (SAD)(Krieger, 1964), and the results have been compared with those of intact rats or each other. Finally, we examined the effect of hypercapnia on baroreflex control of the cardiovascular system by assessing changes in HR in response to an increase or decrease in ABP.

2. Materials and methods

This study was performed in accordance with the guidelines specified for institutional animal care and approved by the ethics committee of animal care and experimentation, University of Occupational and Environmental Health, Japan.

2.1. Animal preparation

All experiments were performed in 37 male Wistar rats, weighing between 400 and 500 g. The animals were divided into six groups: 6 intact, 6 atropine-treated, 6 carotid bodies destroyed (CBD), 6 aortic denervated (AD), 7 carotid bodies destroyed together with aortic denervation (CBAD), and 6 SAD rats. The procedures for preparing the SAD rats and for testing whether SAD was complete have been reported previously (Hirakawa et al., 1997). All procedures were carried out using aseptic techniques. After each operation, the rat was kept under a constant room temperature (25 °C) and received an infusion of Ringer solution containing D-sorbitol and antibiotics.

2.2. Destruction of carotid bodies and transection of aortic nerves

Thirteen rats, initially weighing 250–280 g, were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). After a midline incision was made at the ventral region of the neck, the carotid bifurcation was exposed and both the common and internal carotid arteries were clamped. The carotid body region was then locally frozen by applying a metal rod that had previously been immersed in liquid nitrogen (Verna et al., 1975). Ten weeks after this procedure, 7 of the 13 rats were premedicated with atropine sulfate (0.5 mg/kg, i.p.) and anesthetized with pentobarbital sodium. The carotid bifurcation was again exposed and the aortic and superior laryngeal nerves were sectioned at their junctions with the superior laryngeal and vagus nerves, respectively (Schreihofer and Sved, 1994). All of these procedures were performed bilaterally. Before the implantation of electrodes and catheters, in those rats subjected to transection of the aortic nerves, ABP was allowed to recover for more than 2 weeks to the pre-operation level, like those with SAD (Hirakawa et al., 1997). In rats that had previously been subjected to destruction of the carotid bodies, the carotid baroreceptor function was allowed to recover for more than 3 months (Verna et al., 1975).

2.3. Electrode and catheter implantation

The electrodes utilized for renal sympathetic nerve recording were implanted as described previously, with minor modifications (Nakamura and Hayashida, 1992). Briefly, rats were first anesthetized with pentobarbital sodium (50 mg/kg, i.p.). Through a left flank incision, a branch of the left renal nerve running along or beside the renal artery was exposed and dissected free from the surrounding tissue under a dissecting microscope. Stainless steel bipolar hooked electrodes were used to record renal sympathetic nerve activity (RSNA). The nerve and electrodes were stabilized with a two-component silicone rubber gel (SEMICOSIL 932, Wacker-Chemie, Germany). After the gel hardened, the incision was closed. To record the electrocardiogram (ECG), a pair of Teflon-coated stainless steel wires was implanted under the skin at midchest. Heparin-filled polyethylene catheters were inserted into the abdominal aorta from the right femoral artery to measure ABP. Heparin-filled double-lumen polyethylene catheters were inserted into the inferior vena cava from the right femoral vein to administer drugs. All of the leads and catheters were routed subcutaneously to exit at the nape and protected by a spring coil. The leads were attached to a slip ring so that the rats could move, eat, and drink freely.

2.4. Experimental protocol

More than 4 days after implantation of the electrodes and catheters, each rat was placed in an airtight acrylic chamber (40×25×40 cm) with three holes. One hole, located at the top of a side wall of the chamber, was connected to a multi-flowmeter (model 1203, KOFLOC,

Japan) and used to deliver a gas mixture (air, N_2, and CO_2: total 20 l/min) into the chamber. The second hole was located at the bottom of the opposite wall of the chamber, and was used to flush out the gas mixture. The third hole, located at the top of the chamber, was used to exteriorize the catheters and electrodes. All of the rats were allowed 30–60 min to adjust to their environment in the acrylic chamber ventilated by air, and control measurements commenced when stable ABP, HR, and RSNA were observed. After control measurements were taken for 20 min, air was replaced by a hypercapnic gas mixture (6% CO_2) for 20 min. To avoid hyperoxia due to tachypnea, the O_2 concentration in the chamber was decreased to 16%. The flow of air, N_2, and CO_2 was regulated by a multi-flowmeter, and the O_2 and CO_2 concentrations within the chamber were monitored with a gas analyzer (Respina IH 26, San-ei, Japan). The hypercapnic gas mixture was then replaced by air for a recovery period of 20 min. The experiment was performed between 09:00 and 16:00. The temperature within the chamber was maintained at 25–26 °C.

To assess the influence of the parasympathetic nervous system, atropine methyl nitrate (4.0 mg/kg dissolved in 0.2 ml of saline) was administered intravenously 5 min before the experiment. Atropine was administered in a 0.2-ml volume over a period of 2 min. The dose was determined in accordance with a previous report, with a slight modification (Shirasaka et al., 1999). After the experiment, abolishment of the rapid decrease in HR in response to an injection of phenylephrine (PE) (4 μg/kg, i.v.) was confirmed, indicating that atropine methyl nitrate had completely blocked the parasympathetic effect.

2.5. Blood sampling

For the analysis of arterial blood gases, 0.08 ml of blood was drawn during the control period and again at 15 min after the start of hypercapnic exposure, since ABP and HR had reached a steady state within 15 min during hypercapnic hypoxia in our previous study (Hirakawa et al., 1997; Hirakawa and Hayashida, 2002). Blood samples were analyzed for pH, arterial partial pressure of O_2 (PaO_2), and arterial partial pressure of CO_2 ($PaCO_2$) by a gas analyzer (ABL-520, Radiometer, Denmark).

2.6. Relationship between MABP and HR

To examine the relationship between mean ABP (MABP) and HR, ramped increases and decreases in MABP were performed before and during hypercapnic exposure. The ramp increase in MABP (0.32–0.80 mm Hg/s) was produced by infusion of PE (0–1.0 ml/min of 20 μg/ml) and the ramp decrease in MABP (0.47–0.96 mm Hg/s) was produced by infusion of nitroprusside (NP) (0–1.0 ml/min of 100 μg/ml), using an infusion pump (CMA/100, CMA/Microdialysis, Sweden). All rats were subjected to hypercapnic conditions for 15 min. PE or NP was then administered in a random order with an inter-administration interval of at least 10 min, during which MABP, HR, and RSNA returned to their pre-administration levels. The analog signals of MABP and HR were digitized every 1.4 ms and averaged every second. The data relating HR to the increase or decrease in MABP were subjected to a logistic function using an equation based on the mathematical model described by Kent et al. (1972): $HR=P4+P1/\{1+\exp[P2(MABP-P3)]\}$, where P1 is the range of HR (maximum value–minimum value), P2 is the slope coefficient, P3 is MABP at the midrange of the curve, and P4 is the minimum HR. Baroreflex sensitivity (BRS) was defined as the maximum gain of the curve, which was calculated as $-P1\times P2\times 0.25$.

2.7. Data recording

The original RSNA was amplified and filtered (50–3000 Hz) using a differential low-noise amplifier (AVB-11, Nihon Kohden, Japan) and monitored continuously on an oscilloscope (VC-11, Nihon Kohden, Japan) and an audio speaker. Mean RSNA was obtained by integrating the rectified signal with a time constant of 0.1 s through a voltage integrator (EI-601G, Nihon Kohden, Japan), and rectified RSNA was also obtained using an integrator (EI-600G, Nihon Kohden, Japan). After the experiment, the background noise was determined with nerve activity eliminated by increasing ABP with PE (4–10 μg/kg). The background noise was subtracted from the integrated RSNA data obtained during the experiment. To quantify the RSNA response, the percent change in the response was calculated by taking the mean of these values during the control period to be 100% RSNA. HR was obtained from a cardiotachometer triggered by the R wave of the ECG signal. For subsequent analysis, the analog outputs of RSNA, integrated RSNA, ABP, HR, and ECG were recorded on a digital tape recorder (PC116, SONY, Japan), and also fed to an analog-to-digital converter (PowerLab, ADInstruments, USA). These values were displayed using a Macintosh microcomputer (Power Mac G4, Apple Computer, USA) and saved on a disk.

We extracted fluctuating respiratory changes in ABP by eliminating other fluctuations, such as those originating from cardiac activity and/or Mayer waves (Daly, 1986), using a narrower band-pass filter (E-3201A, NF Electronic Instruments, Japan) with an attenuation ratio of more than one-third per octave. For extraction, the center frequency of the filter was manually adjusted to the actual respiratory frequency between 1 and 2.5 Hz from the preset frequency so that smooth and large waves could be obtained. The respiratory frequency derived by this method has been shown to correspond to the directly recordable respiratory chest movement (Nakamura et al., 1996).

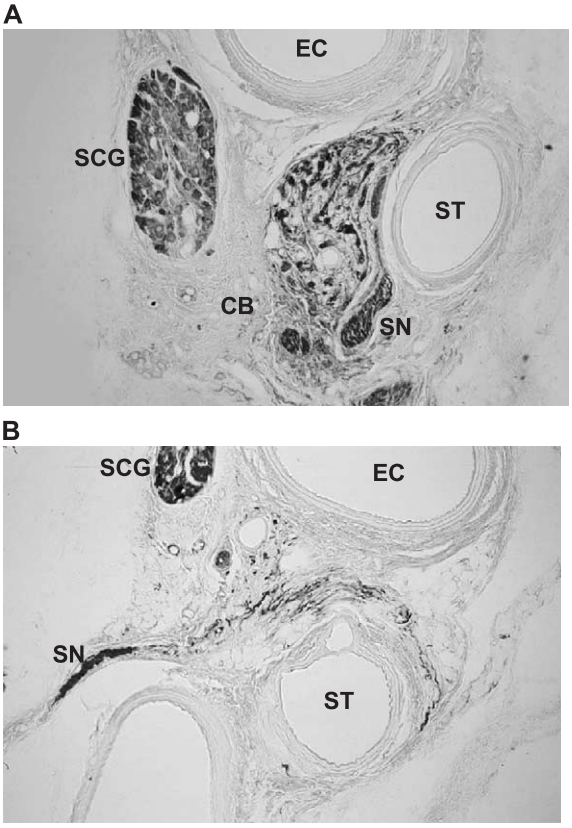

Fig. 1. (A) Section of carotid body bifurcation stained with tyrosine hydroxylase antiserum (TH) from an intact rat. (B) Section of carotid bifurcation stained with TH from a rat that had undergone carotid body lesion by freezing 3 months previously. CB, carotid body; EC, external carotid artery; SCG, superior cervical ganglion; SN, sinus nerve; ST, superior thyroid artery.

2.8. Histological examination

To confirm the destruction of the carotid body by freezing, we performed immunohistochemical staining with tyrosine hydroxylase (TH) antiserum. After the final experiment, the rats were intraperitoneally anesthetized with sodium pentobarbital (50 mg/kg), and perfused through a thin nylon tube inserted into the ventricle with 0.1 M heparinized phosphate-buffered saline (PBS), followed by freshly prepared Zamboni's fixative solution (0.2% picric acid and 4% paraformaldehyde in 0.1 M PBS) at a constant flow rate. Both carotid bodies were then removed under a dissecting microscope, and immersed in the same fixative for an additional 6–8 h at 4 °C. After a brief washing in PBS, the specimens were transferred to 30% sucrose in PBS at 4 °C for 24 h. The specimens were cut serially at 15 μm on a cryostat, and mounted in four series on poly-L-lysine-coated slides. The sections were processed for immunohistochemistry using the peroxidase-antiperoxidase (PAP) method. Prior to PAP treatment, sections were dipped in a fresh 0.3% solution of hydrogen peroxide in methanol for 30 min at room temperature to inhibit endogenous peroxidase activity. After being washed in several changes of 0.3% Triton-X in 0.1 M PBS (PBST), the sections were treated for 30 min with a protein-blocking agent (Immunon, PA, USA) at room temperature to block nonspecific protein binding sites. The sections were incubated with antiserum for TH (1:200; Chemicon, CA, USA) to make evident the total population of glomus cells. The antiserum was diluted with 0.2% bovine serum albumin, 1% normal goat serum, and 0.2% sodium azide in PBST. After being rinsed in several changes of PBST, the sections were transferred for 2 h to anti-rabbit IgG (Organo Technica, NC, USA) diluted to 1:200 with 0.2% bovine serum albumin, 1% normal goat serum, and 0.2% sodium azide in PBST at room temperature. The sections were then rinsed with several changes of PBS, transferred for 2 h to rabbit PAP (Jackson Immuno Research, West Grove, USA) diluted to 1:200 with 0.2% bovine serum albumin and rinsed in several changes of PBS. The immunostaining procedure has been previously described in detail (Kusakabe et al., 2002).

2.9. Termination of experiments

At the end of the experiments, the animals that were not used for histological examination were euthanized by intravenous injection of an over dose of pentobarbital.

Table 1
Arterial blood pH, $PaCO_2$ and PaO_2, and the respiratory rate during control and hypercapnic periods

	pH	$PaCO_2$ (mm Hg)	PaO_2 (mm Hg)	Respiratory rate (breaths/min)
Intact rats (n=6)				
Control	7.47±0.01	39.2±0.5	97.8±0.9	76±5
Hypercapnia	7.36±0.01*	54.5±0.7*	93.8±2.2	122±8*
Atropine-treated rats (n=6)				
Control	7.48±0.01	42.9±1.7	97.8±2.8	83±4
Hypercapnia	7.35±0.01*	58.5±2.2*	98.4±2.5	121±6*
CBD rats (n=6)				
Control	7.47±0.01	42.3±1.3	94.7±1.7	75±4
Hypercapnia	7.36±0.01*	56.8±1.3*	96.6±0.9	119±6*
AD rats (n=6)				
Control	7.49±0.01	39.4±0.6	95.1±1.7	74±4
Hypercapnia	7.38±0.01*	56.5±1.0*	99.1±4.0	117±5*
CBAD rats (n=7)				
Control	7.48±0.01	40.2±1.1	89.8±4.1	74±2
Hypercapnia	7.36±0.02*	56.8±0.7*	99.1±2.0*	117±5*
SAD rats (n=6)				
Control	7.46±0.01	42.0±1.5	93.9±3.8	73±1
Hypercapnia	7.34±0.02*	63.1±5.1*	101.7±3.4*	101±3*

Values are means±S.E. of mean arterial blood pH, PCO_2 ($PaCO_2$), PO_2 (PaO_2). CBD, carotid bodies destroyed; AD, aortic denervated; CBAD, carotid bodies destroyed and aortic denervated; SAD, sinoaortic denervated.

* $P<0.05$ vs. each control.

2.10. Statistical analysis

Between-group and within-group comparisons were performed using a two-way analysis of variance (ANOVA). In ANOVA, when the F-values were significant ($P<0.05$), individual comparisons were made using Fisher's least significant difference test. All data are expressed as means±S.E.

3. Results

3.1. Verification of carotid body destruction

Fig. 1 shows the carotid bifurcation immunostained with TH antiserum from intact (A) and carotid body destroyed (B) rats. The carotid body (glomus cell) is easily recognizable by its location between the superior thyroid artery (ST) and superior cervical ganglion (SCG) in Fig. 1A, and a number of TH-immunostained glomus cells are observable. Regenerated sinus nerve (SN) and the absence of glomus cells may be seen between the ST and SCG in Fig. 1B. Freezing procedure was successful in 13 out of 20 rats.

3.2. Blood gas analysis and respiratory rate

Table 1 shows the mean values for pH, $PaCO_2$, PaO_2, and the respiratory rate during the control and hypercapnic periods in intact (n=6), atropine-treated (n=6), CBD (n=6), AD (n=6), CBAD (n=7), and SAD (n=6) rats. In all groups, there was a significant decrease in arterial pH and a significant increase in $PaCO_2$ compared to the control, but there was no significant change in PaO_2. The respiratory rate increased in all groups and there were no significant

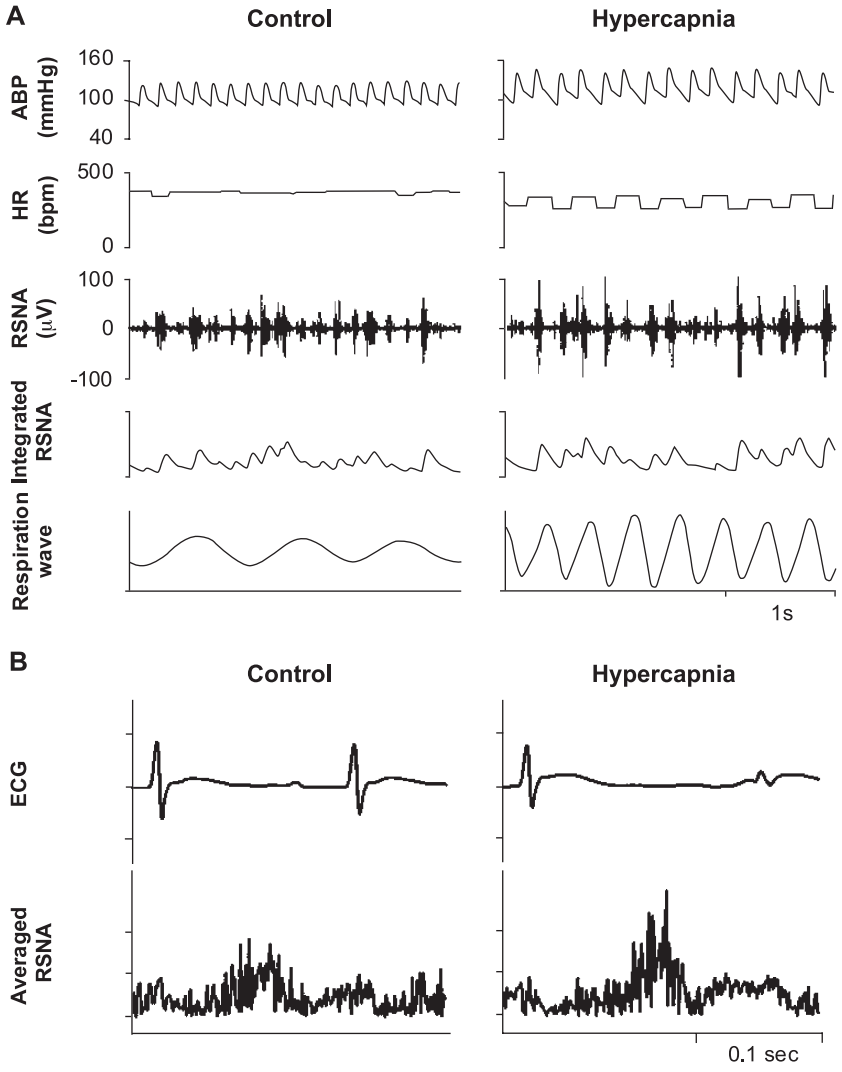

Fig. 2. (A) Typical recordings of arterial blood pressure (ABP), heart rate (HR), original neurogram of renal sympathetic nerve activity (RSNA), integrated RSNA, and respiratory wave form during the control (left) and hypercapnic periods (right) in an intact rat. (B) Averaged sympathetic nerve activities of 32 sweeps triggered by the R-wave of the ECG during the control and hypercapnic periods in an intact rat.

differences in pH, $PaCO_2$, PaO_2 or the respiratory rate between any of the groups during the control period or during hypercapnia.

3.3. Responses to hypercapnic exposure in intact and atropine-treated rats

Fig. 2A shows typical recordings for ABP, HR, RSNA, and the respiration wave form during the control and hypercapnic periods in an intact rat. Hypercapnia increased MABP from 105 to 117 mm Hg, RSNA from 100% to 115%, and the respiratory rate from 63 to 151 breaths/min, but decreased HR from 374 to 302 beats/min. Fig. 2B shows the average sympathetic nerve activities triggered by the R-wave of the ECG during the control and hypercapnic periods in an intact rat. Hypercapnia induced an increase in amplitude of RSNA synchronized with the cardiac cycle.

Fig. 3 shows the time course of MABP, HR and RSNA in intact (A) and atropine-treated (B) rats during the control, hypercapnic and recovery periods. Mean values for 20 min during the control period and the last 5 min during hypercapnic exposure are also shown in Table 2. MABP and RSNA significantly increased in intact and atropine-treated rats during hypercapnia. HR significantly decreased from 357±7 to 312±10 beats/min ($P<0.05$) in the intact rats but significantly increased from 395±15 to 402±7 beats/min ($P<0.05$) in the atropine-treated rats.

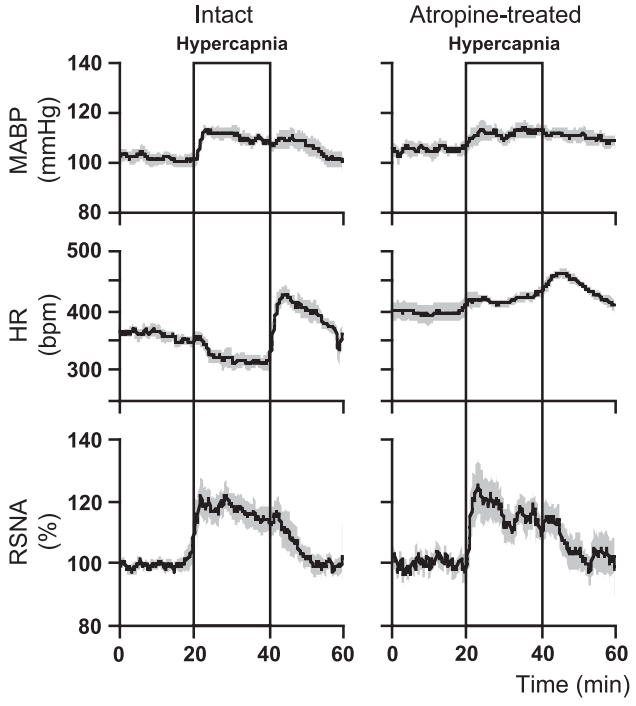

Fig. 3. Mean arterial blood pressure (MABP), heart rate (HR), and renal sympathetic nerve activity (RSNA) during control (0–20 min), hypercapnic (20–40 min), and recovery periods (40–60 min) in intact and atropine-treated rats. Solid lines represent mean values. Shaded areas represent ±S.E.

Table 2
MABP, HR, and RSNA during control and hypercapnic periods

	MABP (mm Hg)	HR (beats/min)	RSNA (%)
Intact rats (n=6)			
Control	101±2	357±7	100
Hypercapnia	108±1*	312±10*	114.6±4.1*
Atropine-treated rats (n=6)			
Control	104±1	395±15†	100
Hypercapnia	112±2*	422±7*,‡	114.4±4.1*
CBD rats (n=6)			
Control	103±1	351±13	100
Hypercapnia	110±1*	312±11*	113.1±2.2*
AD rats (n=6)			
Control	102±2	363±7	100
Hypercapnia	109±3*	363±9‡	116.7±2.6*
CBAD rats (n=7)			
Control	102±2	360±9	100
Hypercapnia	115±2*	367±8‡	116.2±1.5*
SAD rats (n=6)			
Control	98±4	353±6	100
Hypercapnia	105±6*	388±13*,‡	113.3±3.6*

Values are means±S.E. MABP, mean arterial blood pressure; HR, heart rate; RSNA, renal sympathetic nerve activity; CBD, carotid bodies destroyed; AD, aortic denervated; CBAD, carotid bodies destroyed and aortic denervated; SAD, sinoaortic denervated.

* $P<0.05$ vs. each control.

† $P<0.05$ vs. control in intact rats.

‡ $P<0.05$ vs. hypercapnia in intact rats.

3.4. Responses to hypercapnic exposure in CBD (n=6), AD (n=6), and CBAD (n=7) rats

The time course data for MABP, HR, and RSNA before, during and after hypercapnia in CBD, AD and CBAD rats are shown in Fig. 4A, B, and C. Mean values for MABP, HR and RSNA are shown in Table 2. MABP or RSNA significantly increased in the CBD, AD, and CBAD rats. There were no significant differences in MABP or RSNA during the control period between the intact rats and the three chemo-denervated groups. Hypercapnia significantly increased MABP and RSNA in intact, CBD, AD, and CBAD rats. There were also no significant differences in the magnitudes of increase in MABP and RSNA during hypercapnia between the intact and the three chemo-denervated groups. HR did not change in the AD or CBAD rats during hypercapnia, but decreased in the CBD rats at the same magnitude as in the intact rats.

3.5. Responses to hypercapnic exposure in SAD rats (n=6)

The mean values for MABP, HR and RSNA during control and hypercapnia are shown in Table 2. Hypercapnia induced a significant increase in MABP, RSNA and HR (Fig. 4D). There were no significant differences in the

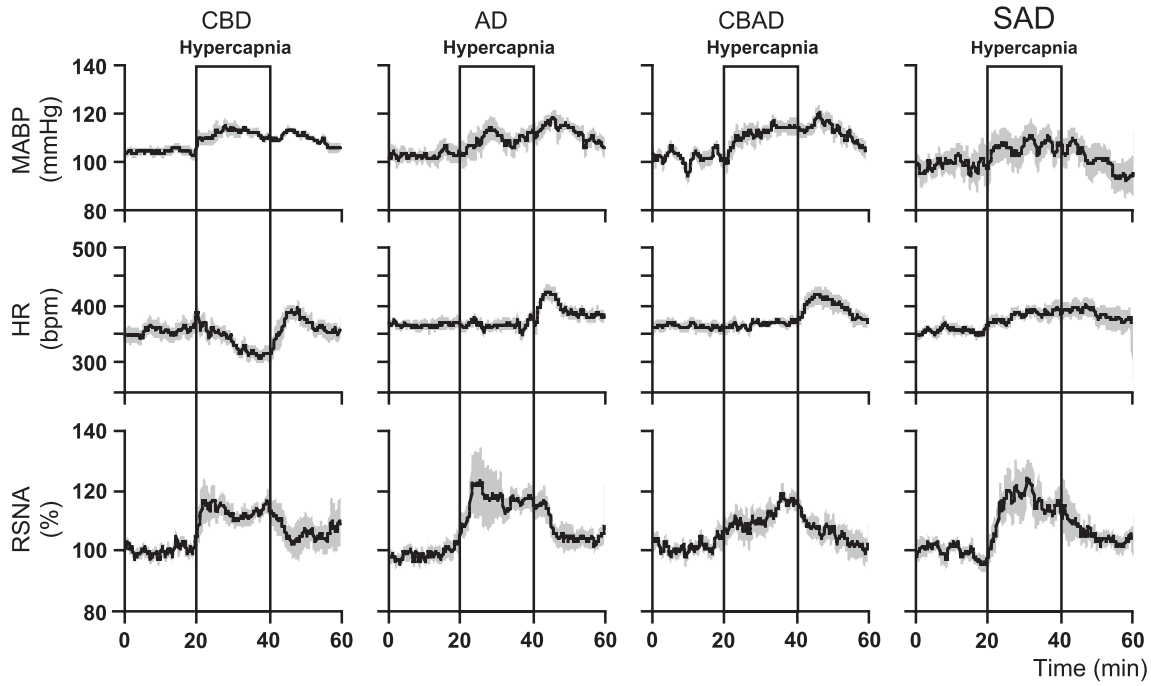

Fig. 4. Mean arterial blood pressure (MABP), heart rate (HR), and renal sympathetic nerve activity (RSNA) during control (0–20 min), hypercapnic (20–40 min), and recovery periods (40–60 min) in carotid bodies destroyed (CBD), aortic nerves denervated (AD), carotid bodies destroyed and aortic denervated (CBAD), and sinoaortic denervated (SAD) rats. Solid lines represent mean values. Shaded areas represent ±S.E.

magnitudes of MABP and RNSA increase between intact and SAD rats.

3.6. Relationship between MABP and HR

Fig. 5 shows the relationships between MABP and HR during the control (A) and hypercapnic periods (B). MABP-HR relationships are summarized in Table 3. There were no significant differences in P1, P3 or P4 between the control and hypercapnic periods in the intact and the three chemo-denervated groups. The slope coefficient (P2) and maximum gain in the AD and CBAD rats were significantly lower than those in the intact group during the control and hypercapnic periods. There were no significant differences in P2 or the maximum gain between the intact and CBD groups, or between AD and CBAD during the control period. Hypercapnia did not affect any of the parameters or the maximum gain in the intact rats, but did

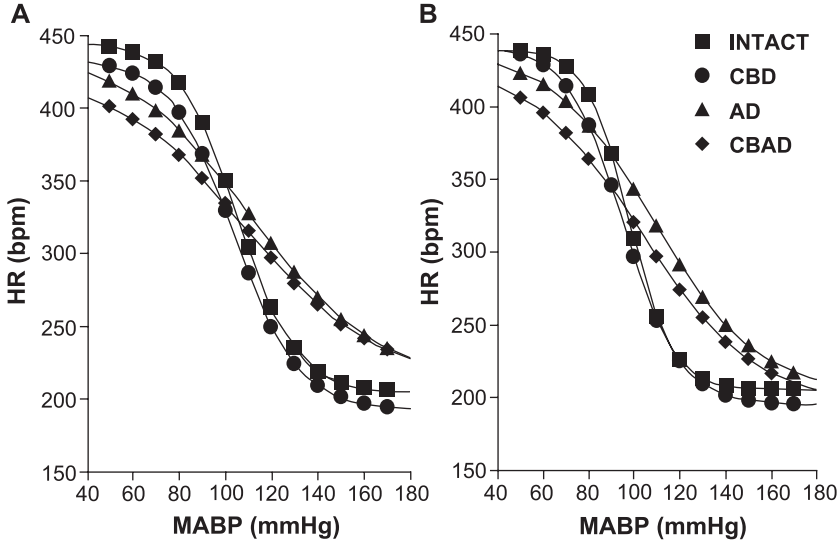

Fig. 5. Relationship between mean arterial blood pressure (MABP) and heart rate (HR) during control (A) and hypercapnic periods (B). CBD, carotid bodies destroyed; AD, aortic denervated; CBAD, carotid bodies destroyed and aortic denervated.

Table 3
Values of P2 in the equation of the baroreflex function and maximum gain during control and hypercapnic periods

	P2	Max. gain (bpm/mm Hg)
Intact rats (n=6)		
Control	0.078±0.017	4.6±1.0
Hypercapnia	0.106±0.018	6.0±0.8
CBD rats (n=6)		
Control	0.072±0.008	4.3±0.5
Hypercapnia	0.081±0.011	4.9±0.6
AD rats (n=6)		
Control	0.037±0.001†	2.1±0.1†
Hypercapnia	0.043±0.003*,‡	2.6±0.2*,‡
CBAD rats (n=6)		
Control	0.036±0.003†	1.9±0.2†
Hypercapnia	0.041±0.002‡	2.4±0.2*,‡

Values are mean±S.E. P2, slope of reflex response; max. gain, maximum gain; bpm, beats/min; CBD, carotid bodies destroyed; AD, aortic nerves denervated; CBAD, carotid bodies destroyed and aortic nerves denervated.
* $P<0.05$ vs. each control.
† $P<0.05$ vs. control in intact rats.
‡ $P<0.05$ vs. hypercapnia in intact rats.

induce slight increases in P2 and maximum gain ($P<0.05$) in both AD and CBAD.

4. Discussion

The major findings in this study in rats are: (1) hypercapnia induces increases in MABP and RSNA, but a decrease in HR in intact rats, (2) while neither atropine treatment nor SAD affects the MABP or RSNA responses, they both induce an increase in HR, (3) bilateral carotid chemo-denervation does not affect either cardiovascular or RSNA responses, (4) bilateral aortic denervation does not affect the MABP or RSNA responses but does abolish the bradycardic response, (5) while the baroreflex control of HR is not affected by carotid chemo-denervation, it can be attenuated by aortic denervation, and (6) hypercapnia exerts no significant effect on the baroreflex control of HR in intact rats.

In this experiment, a novel method of eliminating chemoreceptor elements of the carotid body by localized freezing, while leaving arterial baroreceptor function intact, made it possible to investigate the functional significance of the carotid chemoreceptors. The influence of this surgical procedure on arterial baroreflex function seems to be negligibly small, since there was no difference in the sensitivity of baroreflex control of HR between the intact and CBD rats. This finding is also supported by a previous finding reported by Verna et al. (1975) in the rabbit. They reported that carotid sinus nerve reinnervation was complete after 3 months, as verified by electrophysiological recording of baroreceptor afferent activities. We did not ascertain that whether there were chemoreceptor activities after 3 months after the freezing procedure, but the disappearance of glomus cells has been histologically verified.

The present study is the first to demonstrate that hypercapnia is able to induce increases in MABP and RSNA not only in intact rats, but also in SAD and rats with three types of denervation. There were no significant differences in the magnitudes of the increase in MABP and RSNA during hypercapnia among the intact, SAD, or the three denervated rats. These results suggest that the increase in MABP was mainly due to the sympatho-excitatory effect of hypercapnia via its effect on the central nervous system, and that peripheral chemoreceptors are not essential for the sympathetic nervous system activation in response to hypercapnia. In a previous study, superfusion of hypercapnic solution over the ventral surface of the medulla induced sympatho-excitation (Lioy et al., 1981). There have also been many reports that the increase in ABP seen during hypercapnia is the result of sympatho-excitation, as can be directly observed in recordings from the peroneal nerve (Somers et al., 1989), a branch of the ansae subclaviae (Kollai and Koizumi, 1979), as well as in cardiac and renal sympathetic nerves (Fukuda et al., 1989), and also indirectly by an increase in catecholamines (Rose et al., 1983). Our results also indicate that the known local vasodilator effect of CO_2 is not sufficiently effective as to counteract CO_2-induced sympatho-excitation and vasoconstriction.

In this investigation, hypercapnia induced a decrease in HR and an increase in RSNA in intact rats. Atropine treatment did not alter the RSNA response but elicited an increase in HR. These results suggest the simultaneous activation of the sympathetic and parasympathetic nervous systems during hypercapnia in intact rats. This conclusion is consistent with a previous report that hypercapnia is able to induce increases in both sympathetic and cardiac vagus nerve activity in anesthetized dogs (Kollai and Koizumi, 1979). We have previously shown that this co-activation of the sympathetic and parasympathetic nervous systems resulted in bradycardia during hypercapnic hypoxia (Hirakawa et al., 1997; Hirakawa and Hayashida, 2002). In addition, bradycardia and an increase in sympathetic nerve activity were also reported in response to hypoxic hypoxia in conscious rabbits (O'Hagan et al., 1995).

Hypercapnia produced a decrease in HR and an increase in RSNA in intact rats, and the bilateral destruction of carotid bodies (CBD) did not affect the HR or the RSNA response observed in these animals. Furthermore, hypercapnia did not induce a significant change in HR in either the AD or CBAD rats. There was no difference in the magnitude of increase in RSNA between the AD and CBAD rats. Thus, carotid chemo-denervation did not affect the HR or the RSNA responses to hypercapnia, indicating that carotid chemoreceptors do not play a role in the HR or autonomic nervous response to hypercapnia in rats. This conclusion is consistent with previous results which have shown that carotid chemoreceptors exhibit little, if any,

response to a CO_2 stimulus in rats (Fukuda et al., 1987) or humans (Lugliani et al., 1973).

Hypercapnia induced a decrease in HR in intact and CBD rats, but did not induce any change in HR in rats which had been subjected to aortic denervation (i.e., the AD and CBAD rats). Moreover, the maximum gain in the baroreflex control of HR in the AD and CBAD rats was significantly lower than in the intact rats during hypercapnia. Thus, the observation that aortic denervation attenuated the baroreflex control of HR as well as the bradycardic response, indicates that the afferent input via the aortic baroreceptor is an important factor in determining the hypercapnic HR response. In addition, in SAD rats HR increased, but HR was unchanged in CBAD rats during hypercapnia, suggesting that carotid baroreceptors also contributed to the hypercapnia induced bradycardia seen in conscious rats. Accordingly, afferent input that evokes parasympatho-excitation, through an activation of carotid and aortic baroreceptors, plays an important and perhaps even dominant role in the HR decrease during hypercapnia in intact rats. These suppositions are consistent with a previous report that hypercapnia-induced bradycardia could be abolished by denervation of the carotid and aortic baroreceptors in rats with the carotid bodies left intact (Walker and Brizzee, 1990). The authors of that report concluded that the arterial baroreflex is an important component in determining the overall cardiovascular response to hypercapnia in intact rats. There have also been reports on the direct cardiac depressant effect of hypercapnia on the sinoatrial node (Suutarinen, 1966; Fukuda et al., 1989). However, hypercapnia induced an increase in HR in SAD rats, in which the peripheral baro- and chemoreceptor function had been abolished. This result indicates the direct inhibitory effect of hypercapnia on the heart is negligible in conscious rats.

The present findings suggest that the aortic baroreceptors play an important role in the responses. However, it is not likely that the sustained decrease in HR is mediated only by the arterial baroreceptors, because a rapid decrease in HR in response to an increase in BP is entirely due to an increase in parasympathetic nerve activity, while a sustained decrease in HR is mediated by withdrawal of the sympathetic component (Coleman, 1980). The present study is apparently not the case, since a decrease in HR was sustained in spite of an increase in sympathetic nerve activity. On the other hand, a sustained decrease in HR was observed during the stimulation of the carotid or aortic chemoreceptors (Angell James and Daly, 1969). Accordingly, we ascribed the sustained decrease in HR to the reflex response mediated by aortic chemoreceptors. As far as the rat aortic nerves or aortic bodies are concerned, it has been reported that the rat aortic nerve contains only baroreceptor afferents (Kobayashi et al., 1999) and that the rat aortic bodies were not functioning (Sapru and Krieger, 1977). However, recent anatomical study found the aortic body in rats, as in other mammals, to be composed of a significant amount of glomus tissue, and the aortic nerves to include chemoreceptor as well as baroreceptor fibers (Cheng et al., 1997). In another report, the presence of chemoreceptor tissue in the thoracic region of the rat has been confirmed and chemoreceptor afferents were demonstrated electrophysiologically (Brophy et al., 1999). Thus, it is reasonable to assume that the aortic denervation in this study eliminated not only the aortic baro- but also the chemoreceptor elements, and the possibility that the aortic chemoreceptors may be also responsible for the bradycardic response to systemic hypercapnia cannot be excluded.

It has been established that cardiovascular and autonomic responses are secondarily modulated by changes in respiration (Daly, 1986). Hypercapnia induced a large increase in respiratory rate. Because the RSNA couples with the respiratory cycle (Nakamura et al., 1996), together with the increase in the amplitude of RSNA synchronized with the cardiac cycle, the hyperventilation in response to hypercapnia may relate to the increase in RSNA. The present study does not allow us to exclude the effect of afferent input from the cardiopulmonary receptors on autonomic and cardiovascular responses to hypercapnia, since the *vagi* were intact. However, it has been suggested that the reflex effect elicited by pulmonary stretch receptor activation, which is strong in the dog, is weak or absent in the rat (Marshall, 1987). There was no significant difference in the respiratory rate and in any of the blood gas values during hypercapnia among the groups in this study. Therefore, it is unlikely that a secondary effect of hyperventilation modulated the HR response in these animals or produced the differences in these responses among the groups during hypercapnia.

We conclude that, in the rat, systemic hypercapnia induces an increase in ABP due to sympatho-excitation via central chemoreceptor activation, and a decrease in HR due to parasympatho-excitation via reflex effect of carotid and aortic baroreceptor activation in response to an increase in ABP. In addition, the carotid chemoreceptors do not play a major role in the cardiovascular response to hypercapnia in the conscious rats. This study also suggests that any afferent inputs though the aortic nerves are important sources of the parasympatho-excitation, in which the aortic chemoreceptors may be involved.

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