The Effect of Vagal Afferent on Total Vascular Compliance in Rats

Takashi KINOSHITA

This study was designed to investigate the effect of vagal afferent stimulation on total vascular compliance (TVC). Rats were anesthetized with sodium pentobarbital and artificially ventilated, TVC was determined together with stressed and unstressed blood volumes by measuring mean circulatory filling pressure (Pmcf) at three different levels of circulating blood volume. Measurements was repeated with the intact vagus, after vagotomy and during stimulation of vagal afferents. Vagotomy caused no change in TVC, Pmcf, and stressed and unstressed blood volumes. On the other hand, electrical stimulation of the vagal afferents for 30 sec increased TVC from 3.03 ± 0.51 to 3.39 ± 0.44 ml·mmHg^{-1}·kg^{-1} ($P < 0.05$) and decreased Pmcf from 7.85 ± 1.40 to 7.22 ± 1.21 mmHg ($P < 0.05$). Neither stressed nor unstressed blood volume was changed by vagal stimulation. These results indicate that excitation of vagal afferent causes venodilation and increases TVC without changing stressed and unstressed blood volumes. (Key words: vagal afferent, mean circulatory filling pressure, total vascular compliance, stressed blood volume, unstressed blood volume)

(Kinoshita T: The effect of vagal afferent on total vascular compliance in rats. J Anesth 7: 198–205, 1993)

Central venous pressure (CVP) plays important roles in the maintenance of cardiac output[1], and also in the regulation of body fluid via cardiopulmonary receptors[2]. Recently, Takamata et al.[3] have shown that the reduction of CVP increases total peripheral vascular resistance. Thus, it is possible that the changes in CVP or left atrial pressure modify vascular compliance via cardiopulmonary receptors. Furthermore, changes in stressed and unstressed blood volume can modify CVP[1], but quantitative analysis of the interaction between neural input from cardiopulmonary receptors and total vascular compliance (TVC) or stressed and unstressed blood volumes have been scarcely studied.

To modify the input to cardiopulmonary receptors, head-out water immersion[2] and lower body negative pressure[4] have been used. However, these maneuvers can not be used to study changes in TVC, because they inevitably cause blood shift and modify CVP and stressed and unstressed blood volume. Thus, in this study we analyzed the changes in TVC and in stressed and unstressed blood volumes with the vagus nerve intact, after vagotomy and during electrical stimulation of vagal afferents. The TVC was determined by measuring mean circulatory

Department of Anesthesiology, Kyoto Prefectural University of Medicine, Kyoto, Japan

Address reprint requests to Dr. Kinoshita: Department of Anesthesiology, Kyoto Prefectural University of Medicine, 465 Kajiicho, Kamigyoku, Kyoto, 602 Japan

filling pressure (Pmcf) with different circulating blood volumes (CBV).

Methods

Material and general procedure

Rats were anesthetized with sodium pentobarbital, (initial dose 70 $mg{\cdot}kg^{-1}$, and with a maintenance dose of 7 $mg{\cdot}kg^{-1}$). A tracheal cannula was inserted and the carotid arteries and vagi on both sides were dissected from the neck and separated from each other. Each rat was artificially ventilated with oxygen-enriched air (F_{IO_2}=0.4–0.7) and paralyzed with pancuronium bromide (initial dose 3 $mg{\cdot}kg^{-1}$, maintenance dose 1 $mg{\cdot}kg^{-1}$). The additional administrations of sodium pentobarbital and pancuronium bromide were made at least 10 min before measurements with the intact vagus, after vagotomy, and during vagal afferent stimulation. We confirmed the retrieval of arterial blood pressure (MAP) to the preexisting level. The tidal volume was set to 1.5–2 ml and the frequency was 60 $breaths{\cdot}min^{-1}$.

Map was measured from a PE-50 tube placed in the descending aorta via the right femoral artery. CVP was measured from a PE-50 tube advanced into the inferior vena cava from the left femoral vein. These catheters were connected to strain gauge pressure transducer (P23 ID, Statham-Gould, Instrument Division, Cleveland) and pressures were measured with a strain amplifier (Nihondenki Sanei, 6M62, Tokyo) and recorded on a thermal recorder (Nihondenki Sanei, Recti-horiz-8K, Tokyo). In addition to these catheters, a PE-50 catheter was inserted into the inferior vena cava from right femoral vein to modify blood volume in protocol A. In protocol B, PE-50 catheters were inserted into the inferior vena cava and the descending aorta from the right femoral vein and artery to establish an arteriovenous shunt for measurement of CBV.

Stimulation of vagal afferent

The proximal cut end of the right vagus nerve was fixed on a bipolar Ag-AgCl electrode and electrical stimuli of 100 Hz and 7V with a pulse duration of 2 ms were applied (Nihonkoden, SEN-2201, Tokyo) for 30 sec starting 20 sec before the measurement of Pmcf. When blood volume was changed, infusion or withdrawal was started 10 sec after the beginning of stimulation. After each experiment, the stimulated right cervical trunk was examined with dissecting microscope. Data from animals in which the right vagus had no cardiac branch were discarded.

Measurement of Pmcf and TVC

Pmcf was measured by the method developed by Yamamoto[5]. A 3 Fr. catheter (Disposable Indwelling Tube For Infant Feeding, Atom, Tokyo) with an inflatable latex balloon was inserted into the left external jugular vein and advanced into the right atrium. The balloon was inflated for about 7–8 sec to reduce cardiac output to zero. Then the venous plateau pressure (VPP) and the final arterial pressure (FAP) were measured as shown in figure 1. Pmcf was calculated according to the equation

$$\text{Pmcf} = \text{VPP} + 1/60\ (\text{FAP-VPP})^{6},$$

assuming arterial-to-venous capacitance ratio as 1/60[5]. This measurement was repeated during hypovolemia, normovolemia, and hypervolemia, with intervals of at least 10 min between measurements. To change the blood volume, we infused or withdrew blood amounting 10 $ml{\cdot}kg^{-1}$. The infused blood was obtained from an donor rat.

TVC was computed from the changes in blood volume and the concomitant changes in Pmcf as the slope of a 3-point linear regression by least square analysis.

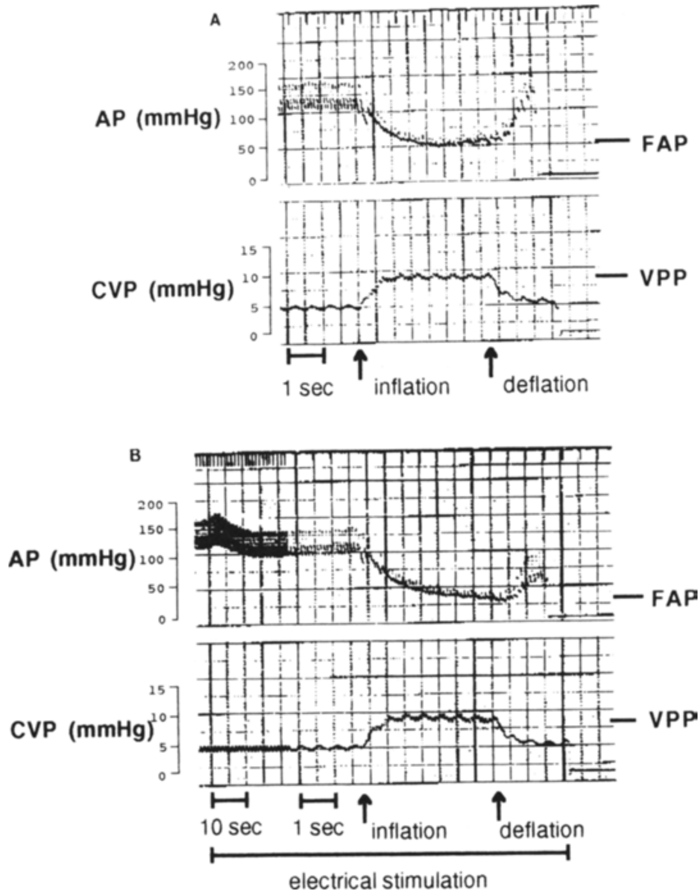

Fig. 1. A typical recording used to measure final arterial pressure (FAP) and venous plateau pressure (VPP). The upper panel shows arterial pressure, and the lower panel shows central venous pressure, during balloon inflation. "A" shows a typical recording of arterial and central venous pressures under normal conditions

"B" during vagal electrical stimulation.

The Pmcf/blood volume relationship was extrapolated to the volume-axis intercept and the CBV at zero Pmcf was used as the unstressed blood volume. Stressed blood volume was obtained as the difference between CBV and the unstressed blood volume.

Experimental procedures

Protocol A: Measurement of Pmcf with the intact vagus, after vagotomy, and during vagal afferent stimulation.

Experiments were performed on 13 male Wistar rats (362 ± 50g, mean ± SD). Pmcfs were measured with the intact vagus during normovolemia, hypervolemia and hypovolemia in the order. Pmcf was measured 20 sec after the end of blood infusion or withdrawal, and then CBV was immediately restored to the previous level. The total time required to change the CBV, measure Pmcf, and restore the animal to normovolemia was about 40 sec.

At least 60 min after vagotomy Pmcf was measured at each of the three levels of CBV. Then, Pmcf was measured during electrical stimulation of the vagi at each of the three levels of CBV, as described above. Pmcf was measured 30 sec after the start of vagal electrical stimulation and 10 sec after the end of the change in CBV. After measurement of Pmcf, vagal electrical stimulation was stopped.

Protocol B: Measurement of CBV with the intact vagus, after vagotomy, and during vagal afferent stimulation.

In 7 rats, CBV was measured by the

Table 1. Hemodynamic variables in anesthetized rats with the intact vagus, after vagotomy and during vagal electrical stimulation

	Intact vagus	After vagotomy	Electrical stimulation
Pmcf (mmHg)	8.13 ± 1.64	7.85 ± 1.40	7.22 ± 1.21*
TVC ($ml{\cdot}mmHg^{-1}{\cdot}kg^{-1}$)	3.08 ± 0.70	3.03 ± 0.51	3.39 ± 0.44*
CVP (mmHg)	3.0 ± 1.2	3.1 ± 1.1	2.9 ± 1.0
MAP (mmHg)	127.7 ± 14.9	109.9 ± 21.3	96.0 ± 21.3*

Values are means ± SD for 13 animals. Pmcf, mean circulatory filling pressure; TVC, total vascular compliance; CVP, centralvenous pressure; MAP, mean arterial pressure, $^*P < 0.05$ vs. intact vagus.

Table 2. Blood volume along the time course of experiment in anesthetized rats

	Intact vagus	After vagotomy	Electrical Stimulation
CBV ($ml{\cdot}kg^{-1}$)	51.3 ± 3.0	51.2 ± 1.7	51.1 ± 1.7
CVP (mmHg)	2.9 ± 1.3	2.8 ± 1.8	3.1 ± 1.3
BP (mmHg)	125.4 ± 20.4	129.2 ± 24.8	91.9 ± 24.6*

Values are means ± SD for 5 animals. $^*P < 0.05$ vs. intact vagus. CBV, circulating blood volume; CVP, central venous pressure; MAP, mean arterial presure.

dilution of ^{51}Cr with the intact vagus, after vagotomy and during electrical stimulation of the afferent vagus nerve.

Blood volume was continuously monitored as previously described[7]. One milliliter blood was removed from an ether-anesthetized donor rat via cardiac puncture. The cells in this blood were labeled with ^{51}Cr (100 μCi). An arterio-venous extracorporeal shunt was made from the left femoral artery to the left femoral vein. The shunted blood was led by a pump (Watson Marlow, 202U/1, Conwall) to a glass coil placed in a γ-scintillation counter (Osaka Denpa, Osaka), at a rate of 1 $ml{\cdot}min^{-1}$. The blood was infused through the extracorporeal circuit. The CBV at time t was calculated as

$$CBV(t) = V(0) \times C(0)/C(t-t_1) - V_S$$

where $V(0) \times C(0)$ is the total radioactivity count of labeled blood initially injected, $C(t-t_1)$ is the count of shunted blood at time t after corrections for background counts and the time lag of the system (t_1=0.5 min), and V_S is the volume of blood in the shunt (1.73 ml).

Data analysis

Mean values ± SD are presented. The Pmcfs and compliances measured before and after vagotomy, and with electrical stimulation were analyzed by paired t-test. The null hypothesis was rejected when $P < 0.05$.

Results

Typical recordings used to measure Pmcf are shown in figure 1. Pmcf, TVC, CVP and MAP with the intact vagus, after vagotomy and during vagal afferent stimulation are shown in table 1.

Table 3. Stressed and unstressed blood volumes

	Intact vagus	After vagotomy	Electrical Stimulation
CBV ($ml \cdot kg^{-1}$)	51.3 ± 3.0	51.2 ± 1.7	51.1 ± 1.7
USBV ($ml \cdot kg^{-1}$)	30.2 ± 2.5	32.0 ± 4.8	30.1 ± 5.1
SBV ($ml \cdot kg^{-1}$)	21.1 ± 2.5	20.0 ± 4.8	21.0 ± 5.1

Values are means ± SD. CBV, circulating blood volume; USBV, unstressed blood volume; SBV, stressed blood volume.

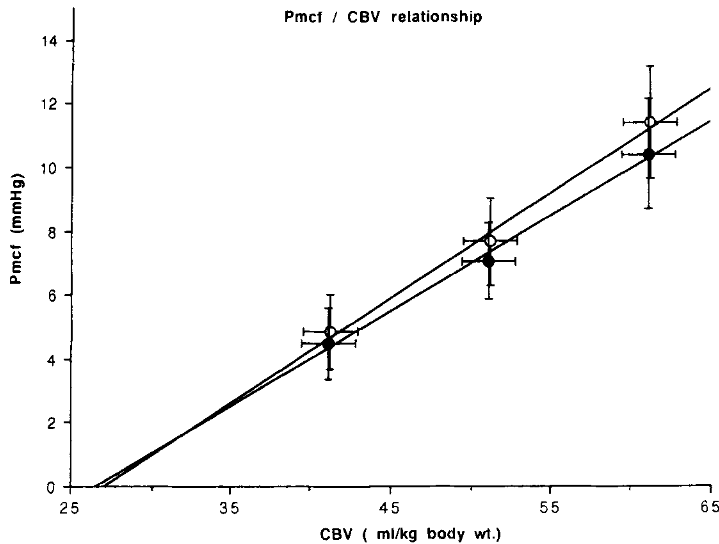

Fig. 2. The relationship between circulating blood volume (CBV) and mean circulatory filling pressure (Pmcf) of rats under normal conditions (open circles) and during electrical stimulation of vagal afferents (closed circles). The error bars show S.Ds.. Fitted lines were obtained from averages of Pmcf at 3 different blood volumes and averages of CBVs in other rats. The X-axis intercepts of the fitted lines indicate unstressed blood volumes. The inverse slopes of the fitted lines represent total vascular compliances.

No significant differences in Pmcf, TVC, CVP or MAP resulted from vagotomy. With vagal afferent stimulation, Pmcf decreased from 7.85 ± 1.40 to 7.22 ± 1.21 mmHg ($P < 0.05$), TVC increased from 3.03 ± 0.51 to 3.39 ± 0.44 $ml \cdot mmHg^{-1} \cdot kg^{-1}$ ($P < 0.05$), and MAP decreased from 109.9 ± 21.3 to 96.0 ± 21.3 mmHg ($P < 0.05$). CVP did not significantly change. Blood volumes obtained with protocol B are shown in table 2 together with CVP and MAP. There were no significant differences in CVP or MAP among the three conditions studied in protocol A. Based on the results of protocols A and B, the stressed and unstressed blood volumes were computed, and the results are shown in table 3. The relationship between averages of Pmcfs at 3 different blood volumes and averages of CBVs are shown in figure 2. Stressed blood volumes with the intact vagus, after vagotomy and during vagal

stimulation were 21.1 ± 2.5, 20.0 ± 4.8 and 21.0 ± 5.1 ml·kg^{-1}, respectively, and there were no significant differences among them. Unstressed blood volumes with the intact vagus, after vagotomy and during vagal stimulation were 30.2 ± 2.5, 32.0 ± 4.8 and 30.0 ± 5.1 ml·kg^{-1}, respectively, and there were no significant differences among them.

Discussion

The changes in cardiac output mainly occur for three reasons: The altered pumping capacity of the heart per se, changes in afterload and cardiac filling pressure or preload[1]. Studies on preload is rather scarce compared to the extensive studies on cardiac contractility and afterload, and in this experiment, feedback regulation of preload or central venous pressure (CVP) was studied.

CVP is regulated by the continuous adjustment of blood volume to the changing size of the vascular bed. The size of vascular bed can be expressed by vascular compliance, and the volume of blood contained in the vascular space includes both the stressed and unstressed blood volumes. The volume of blood in a vessel at zero transmural pressure is defined as the unstressed blood volume, and the volume of blood that must be removed from the vasculature to decrease the transmural pressure of the vessel from the existing value to zero is called the stressed blood volume[8]. Thus, CVP is modified by the changes occuring in the stressed blood volume, unstressed blood volume and vascular compliance under the condition of constant cardiac output.

In this experiment vagal afferent nerve was stimulated simulating loading of cardiopulmonary receptor, and change in CVP was assessed together with changes in vascular compliance, stressed blood volume and unstressed blood volume. For this purpose, Pmcfs at three levels of blood volume were determined, and vascular compliance was obtained from the slope of Pmcfs vs CBVs. Extrapolation of the regression line to zero pressure provides unstressed blood volume and stressed blood volume was obtained from the difference between measured blood volume and unstressed blood volume.

The redistribution of blood volume by application of negative pressure to the lower body[4], head-out water immersion[2] or blood volume modification, has been used to load or unload cardiopulmonary receptors. These methods are not suitable for the present study because these maneuver can change TVC. To induce purely neurogenic changes, we used electrical stimulation of vagal afferents.

The maximum rate of the discharge of atrial receptors has been reported as 55 ± 3 Hz, corresponding to a 20 mmHg increase in left atrial pressure[9] and it is also reported that 92% of vagal afferent fibers in rats are C-fibers[10] and that any ventricular receptors with non-medullated afferent or any medullated afferent could not be localized[9]. Because the duration of non-medullated fiber oriented stimulation is 2 ms[11], stimulation at 100 Hz for 2 ms was used in this experiment. We assume that this procedure can stand for an acute elevation of CVP or left atrial pressure up to supramaximal level.

To stimulate the afferent limb of the vagus nerves, it is necessary to dissect the nerves because stimulation of efferent pathway has considerable inhibitory effects on the heart. Vagotomy causes an unloading of atrial receptor. If unloading of atrial receptor elicit reflex to maintain venous return, vagotomy could result in a lower TVC. However, we found no reduction of TVC, which coincides with the finding

by Shoukas et al[12]. Thus, the effect of vagotomy on TVC can be disregarded, and as far as TVC and unstressed blood volume are concerned the vagotomized animals were equivalent to those with intact vagus and sympathetic nerves.

The effect of the input from cardiopulmonary receptor has been sparsely studied. Ruten suggested that an elevation of left atrial pressure for 4 min caused decrease in intravascular volume[13] and the mean blood volume decrease was approximately 2.7 $ml \cdot kg^{-1}$. Because we measured Pmcfs 90 sec after the start of stimulation, transvascular fluid shift and blood volume change should be minimal. Increased total vascular compliance and decreased Pmcf causes trans-vascular fluid shift from perivascular area to intravascular area[8]. This fluid shift should cause the increase of stressed blood volume and Pmcf. Thus our data represents acute phase of response and should reflect pure vascular response.

In our experiment, vagal afferent stimulation decreased MAP by 18% in protocol A and 20% in protocol B. The hypotension consequent to vagal afferent stimulation might cause vasoconstriction and venoconstriction by carotid baroreflex. Shoukas et al. demonstrated that there was a significant increase in TVC of 12%[14] or 22%[15] immediately after carotid sinus pressure was increased from 50 mmHg to 200 mmHg. In our result an increase in TVC of 18% with vagal afferent stimulation was observed although MAP decreased significantly by 13.9 mmHg. The result suggests that the increase in CVP or hypervolemia is a potent stimuli for the regulation of TVC.

The shift of blood volume between stressed and unstressed blood volume can modify Pmcf and also CVP. In the present experiment, both stressed and unstressed blood volume showed no change during the vagal afferent stimulation, while TVC was increased. The increase in TVC under the condition of increased CVP or cardiac return is advantageous for the feedback regulation of CVP and cardiac output[16].

Recent report by Takamata et al.[3] demonstrated that the reduction of CVP induced by cutaneous vasodilation under hyperthermia increased total peripheral vascular resistance. In addition, the present result shows that the vagal afferent stimulation or the increase in CVP causes venodilation. Thus, it is suggested that CVP plays important roles for feedback regulations in both pre- and after-load.

In summary, we have shown that afferent vagal nerve plays a role to regulate TVC during early phase of hypervolemia before changes in stressed or unstressed blood volume take place.

Acknowledgement: The authors are grateful to Professor T. Morimoto and Professor Y. Tanaka for their supports and valuable discussions.

This study was supported in part by Grant-in-Aid for Scientific Research from The Ministry of Education, Science and Culture, Japan.

(Received Dec. 9, 1991, accepted for publication Jul. 16, 1992)

References

1. Guyton AC: Venous return, Handbook of Physiology. Sect. 2, Vol. 2, Circulation. Edited by Hamilton, WF. Bethesda, American Physiological Society, 1963, pp. 1099–1233
2. Gauer OH, Henry JP: Neurohormonal control of plasma volume, International Review of Physiology, Cardiovascular Physiology II. Edited by Guyton, AC. Baltimore, University Park Press, Vol. 9, 1976, pp. 145–190.
3. Takamata A, Nose H, Mack GW, et al: Control of total peripheral resistance during hyperthermia in rats. J Appl Physiol 69:1087–1092, 1990
4. Brown E, Goei JS, Greenfield ADM, et al: Circulatory responses to stim-

ulated gravitional shifts of blood in humans induced by exposure of the body below the iliac crests to subatmospheric pressure. J Physiol London 183:607–627, 1963
5. Yamamoto J, Trippodo NC, Ishise S, et al: Total vascular pressure-volume relationship in the conscious rat. Am J Physiol 238:H823–H828, 1980
6. Samar RE, Coleman T: Measurement of mean circulatory filling pressure and vascular capacitance in the rat. Am J Physiol 234:H94–H100, 1978
7. Tanaka Y, Morimoto T, Miki K, et al: Online control of circulating blood volume. Jpn J Physiol 31:427–431, 1981
8. Morimoto T: Thermoregulation and body fluids: Role of blood volume and central venous pressure. Jpn J Physiol 40:165–179, 1990
9. Thorén P, Noresson E, Ricksten S-E: Cardiac receptors with nonmedullated vagal afferents in the rat. Acta Physiol Scand 107:13–18, 1979
10. Inoue K: Electron microscopic analysis of the vagus nerves in the mice; its fiber constituent, degeneration and relationship with superior cervical ganglion. J Anat Jap 50:1–14, 1975
11. Öberg B, Thorén P: Circulatory responses to stimulation of medullated and non-medullated afferents in the cardiac nerves in the cat. Acta Physiol Scand 87:121–132, 1973
12. Shoukas AA, Sagawa K: Total systemic vascular compliance measured as incremental volume-pressure ratio. Circ. Res. 28:277–289, 1971
13. Ruten DL: Reflex effects of left atrial elevation on total intravascular volume. Am J Physiol 241:H760–H765, 1981
14. Shoukas AA, MacAnespie CL, Brunner MJ, et al: The importance of the spleen in blood volume shifts of the total vascular bed caused by the carotid sinus baroreceptor reflex in the dog. Circ Res 49:759–766, 1981
15. Shoukas AA, Brunner MC: Epinephrine and the carotid sinus baroreceptor reflex. Circ Res 47:249–257, 1980
16. Shigemi K, Morimoto T, Natsuyama T, et al: Regulation of vascular compliance and stressed relaxation by the sympathetic nervous system. Jpn J Physiol 41:577–588, 1991