Circulatory Responses to Graded Stimulation of the Carotid Chemoreceptors in the Dog

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ABSTRACT

The vascular effects of graded stimulation of the carotid chemoreceptors were studied in 13 anesthetized and artificially ventilated dogs, and stimulus-response curves were defined. The carotid bifurcations were isolated and perfused at constant pressure, and the vagi were cut. Autologous blood collected in a reservoir was equilibrated at Po2 varying from 104 to 34 mm Hg, Pco2 from 39 to 81 mm Hg, and pH from 7.46 to 6.87 and was used to stimulate the carotid chemoreceptors. Systemic arterial Po2, Pco2, and pH were kept normal. Reflex increases in aortic blood pressure and in hind-limb perfusion pressure (constant-flow perfusion) were first seen with Po2 of 70 mm Hg or Pco2 > 39 mm Hg. The vascular responses increased linearly with lower Po2 or higher Pco2 until tensions of 34 and 71 mm Hg, respectively, were reached. These stimuli caused a reflex dilatation of the perfused saphenous vein that was also proportional to the degree of stimulation. Increasing the pH of the blood perfusing the chemoreceptors decreased these changes by more than 50%; decreases in pH, with normal Po2 and Pco2, caused marked vascular responses, indicating an important role of pH in the activation of the chemoreflex mechanism.

KEY WORDS chemoreflex hypoxia hypercapnia acidosis
control of blood pressure venodilatation

The response of the carotid chemoreceptors to changes in Po2, Pco2, and pH has been characterized in terms of electrical activity in the sinus nerve (1) and of reflex respiratory effects (2). These responses appear to depend on the intensity of stimulation of the chemoreceptors, and stimulus-response curves have been defined. The effects of the chemoreflex mechanism on the circulation have been studied qualitatively (3-5), and Daly and Scott (6) have stressed the importance of separating the primary vascular responses caused by the chemoreflex itself from those induced by ventilatory changes. However, studies of the cardiovascular effects have always been performed with an intense degree of chemoreceptor stimulation by hypoxia or hypercapnia or with pharmacologic stimuli (nicotine and cyanide). The minimal level of chemoreceptor excitation necessary to cause circulatory changes has not been determined, nor have the vascular responses been correlated with the degree of stimulation. Hence the precise conditions under which the chemoreflex mechanism becomes involved in the control of circulation cannot yet be appreciated.

In the present experiments, the effect of graded stimulation of the carotid chemoreceptors on the circulation was investigated, and stimulus-response curves were derived for changes in Po2, Pco2, and pH in the blood perfusing the carotid bodies. The results demonstrate that, in the anesthetized dog, the chemoreceptors are an important control mechanism in the overall regulation of the circulation and that their vascular effect is proportional to the degree of stimulation.

Methods

Thirteen dogs weighing 25-30 kg were anesthetized with thiopental and chloralose (15 and 60...
mg/kg, respectively) and artificially ventilated at 12–15 cycles/min. Additional doses of chloralose (10 mg/kg) were administered hourly to maintain an even plane of light anesthesia. Before cannulation of the blood vessels, gallamine and heparin (3 mg/kg each) were given intravenously and repeated hourly (1 mg/kg each). A bilateral cervical vagotomy was performed at the level of the cricoid cartilage.

Perfusion of Carotid Bodies.—The two carotid bifurcations were isolated by ligating the internal carotid arteries distal to the sinus, the occipital arteries and their branches distal to the carotid bodies, and the ascending pharyngeal, facial, and external carotid arteries. The excluded carotid bifurcations were perfused with a roller pump, at a flow rate of 125 ± 15 ml/min (circulation time from pump to bifurcations, 15 ± 3 seconds; tube volume, 30 ml), through cannulas in the cephalad portion of the ligated common carotid arteries, using arterial blood from the left common carotid cannulated caudally. Cannulas in the external carotid arteries served to return the blood perfusing the bifurcations to the right jugular vein (Fig. 1).

**FIGURE 1**

Technique for perfusion of isolated carotid chemoreceptors. Blood collected in reservoir is continuously pumped (P₁) through membrane oxygenator for equilibration at desired level of gases. Temperature in reservoir is kept at 37°C by heat exchanger (HE), and PO₂ is continuously monitored by a flow-through polarographic analyzer (P₀₂). This blood is pumped (P₂) to the carotid bodies (CB) through cannulas in common carotid arteries (CC) and returned to reservoir by cannulas in external carotid arteries (EC). Pressure of perfusion is maintained constant by a Starling resistance (SR). Between stimulations, blood from common carotid artery (CCA) perfuses bifurcations and is returned to dog by jugular vein (JV). IC = internal carotid artery, OA = occipital artery, DP = depulsator.

The perfusion pressure was measured in the inflow cannula proximal to the bifurcations and was maintained constant by a Starling resistance in the outflow circuit. Nonpulsatile perfusion pressure was obtained by interposing a depulsator in the inflow circuit to the carotid bifurcations. The pressure in the bifurcations was adjusted to obtain a mean aortic blood pressure similar to that before cannulation and was maintained constant throughout the experiment. The sinus pressure never varied by more than 3 mm Hg during the course of an experiment. The adequacy of isolation of the carotid bifurcations was verified by the effect of stopping the pump and clamping the outflow circuit. A steady decrease of pressure in the bifurcations to 40 mm Hg or less, despite an increase in systemic blood pressure above 200 mm Hg due to the effect of the baroreceptors, was evidence of satisfactory exclusion of the carotid bodies from the systemic circulation and of the absence of any significant arterial inflow from collateral vessels. A higher residual pressure indicated that further dissection of the bifurcations was necessary to ligate the patent arteries. When extensive dissection was needed, the integrity of the carotid bodies was assessed by injecting 1 mg of sodium cyanide directly into the input cannula to the bifurcations. In 6 of 19 dogs, no response was obtained to cyanide and the experiment was terminated.

**Extracorporeal Circuit.**—A total of 400 ml of autologous arterial blood was collected in a reservoir and replaced by infusing the dogs with a similar volume of dextran 40. This blood was circulated with a roller pump through a silicone rubber membrane oxygenator (Travenol Laboratories, Inc., Morton Grove, Ill.) to be equilibrated at desired levels of PO₂ and PCO₂. The temperature of the blood was measured in the reservoir and kept at 37°C by a heat exchanger. A flow-through polarographic analyzer in the outflow line from the reservoir continuously monitored the PO₂ of the blood in the circuit. Adequate mixing of the blood in the reservoir was demonstrated by the absence of changes in PO₂ once equilibration at a given level was achieved.

**Blood Gas Tensions and pH.**—The dogs were artificially ventilated with room air. The tidal volume was adjusted and oxygen was added to maintain the systemic arterial PO₂ between 90 and 140 mm Hg (mean 111 mm Hg) and PCO₂ between 30 and 44 mm Hg (mean 37 mm Hg) throughout the experiment. Bicarbonate, 7.5%, was administered as needed to keep the arterial blood pH between 7.30 and 7.40 (mean 7.34).

The O₂ and CO₂ tensions in the blood of the extracorporeal circuit were varied independently by delivering to the oxygenator gas mixtures precisely adjusted with calibrated gas flowmeters.
Gases obtained from three tanks (one containing 5% CO₂ in air, one 5% CO₂ in N₂, and one 50% CO₂ in air) were mixed to achieve the desired gas tensions in the blood, using a constant gas flow of 2 liters/min through the oxygenator for a total blood flow of approximately 1 liter/min. Separate adjustments of the pH were made by adding the required amount of either bicarbonate or 0.1N HCl to the blood. When the desired levels of Po₂, Pco₂, and pH were reached and stable, blood from the reservoir was used to stimulate the carotid chemoreceptors by shifting the inflow to the pump perfusing the carotid bifurcations. This blood was returned to the reservoir by an alternate outflow circuit, so that no significant transfusion of blood to the animal occurred (Fig. 1). Each stimulation was maintained for 2–4 minutes before returning to the control state, in which the bifurcations were perfused with arterial blood from the dog. Before each test, blood samples were taken from the aorta and from the reservoir to measure the respective Po₂, Pco₂, and pH values.

Measurements.—All pressures were measured with strain-gauge transducers (Statham P23De) and recorded on an ultraviolet Visicorder (Honeywell 1508).

Aortic Blood Pressure.—Mean and pulsatile pressures were measured through a catheter inserted via the right brachial artery.

Hind-Limb Resistance Vessels.—The left hind limb was perfused via the external iliac artery at constant flow with a roller pump, using autologous blood from the terminal aorta. All branches of the terminal aorta as well as the deep circumflex iliac and the deep caudal epigastric arteries on each side were tied, so that the only blood flow to the limb was delivered by the pump. A depulsator and a heat exchanger to keep the blood temperature at 37 °C were interposed in the perfusion line. The perfusion pressure was measured from the arterial line just proximal to the pump. A depulsator and a heat exchanger to keep the respective PCM, Pco₂, and pH were normal (114 mm Hg, 36 mm Hg, and 7.35, respectively). The changes in perfusion pressure of one saphenous vein in eight dogs, the changes in perfusion pressure of one hind limb in seven, and the changes in circulatory pressures were measured while the carotid sinus pressure was maintained constant. The stimulations were performed once the predetermined levels of gas tensions and pH were achieved in the blood of the reservoir. During the control period between stimulations, sufficient time was allowed to permit the vascular pressures to return and stabilize at the control level. A complete curve was obtained in less than 45 minutes. No anesthetic was administered during the determination of a curve; if shifts of more than 10 mm Hg in the base-line pressures occurred, the data were discarded and a new curve was obtained. To construct the stimulus-response curves, the changes in hemodynamic pressures were plotted as percent of the maximal response against the actual level of Po₂ or Pco₂ in the blood perfusing the chemoreceptors. To permit a direct comparison of curves obtained with separate and combined changes in Pco₂ and pH, the maximal response to simultaneously increased Pco₂ and decreased pH was taken as the reference point for the three curves.

Results

Responses to Changes in Po₂.—In eight dogs, graded hypoxic stimulation of the carotid chemoreceptors was performed by decreasing the Po₂ in the blood perfusing the carotid bodies in steps from 104 mm Hg to 34 mm Hg (means); the Pco₂ and pH were kept constant and averaged 36 mm Hg and 7.35, respectively. The systemic arterial Po₂, Pco₂, and pH were normal (114 mm Hg, 36 mm Hg, and 7.35, respectively). The changes in aortic blood pressure were measured in all eight dogs, the changes in perfusion pressure of one hind limb in seven, and the changes in perfusion pressure of one saphenous vein in five. Figure 2 (top) shows a typical record of the vascular responses observed at four O₂ tensions.
Changes in mean aortic pressure and hind-limb and saphenous vein perfusion pressures in response to graded stimulation of carotid chemoreceptors in dog with vagi cut. Systemic arterial \( P_{O_2} \), \( P_{CO_2} \), and pH were normal; carotid sinus pressure was constant. Time of stimulation is indicated by signal at bottom of each record, along with stimulus used. The scales at the left refer to carotid sinus pressure and saphenous vein perfusion pressure. The scales at the right refer to mean aortic pressure and hind-limb perfusion pressure.

The stimulus-response curves for the changes in aortic pressure and hind-limb perfusion pressure are shown in Figure 3 (left top and bottom). The standard errors of the mean values of \( P_{O_2} \) shown on the x-axis were not greater than 1 mm Hg. At \( O_2 \) tensions of 104 and 80 mm Hg there was a slight increase in aortic pressure, but the changes in hind-limb perfusion pressure were not significant. However, the responses observed at these two levels of \( O_2 \) were the same and can be attributed to other factors. In both aortic and hind-limb curves, the inflection occurred at \( P_{O_2} = 80 \) mm Hg, and the responses increased linearly thereafter over the range of \( O_2 \) tensions studied. The slopes of the two curves were similar and showed no evidence of flattening at the lowest \( P_{O_2} \). The maximal increases in aortic pressure and hind-limb perfusion pressure were \( 88 \pm 15 \) and \( 76 \pm 15 \) mm Hg, respectively, at a mean \( P_{O_2} \) of 34 mm Hg. Comparison of these responses by the paired t-test showed no significant difference \((P > 0.05)\). Although the same type of response was observed in each dog, the maximal changes in pressure varied largely between animals \( (\text{range} \ 30-150 \ \text{mm Hg}) \).

Dilatation of the saphenous vein in response to activation of the chemoreflex was observed in all five dogs. In each, the changes in venomotor tone were also proportional to the level of \( P_{O_2} \) \( (\text{Fig. 2}) \). The initial venodilatation occurred at \( O_2 \) tensions of 70-73 mm Hg in four dogs and at 51 mm Hg in one. The dilatation was maximal at \( P_{O_2} \) of 33-43 mm Hg. Since variations in the venomotor tone during each experiment could not be prevented, it was not possible to assess quantitatively the magnitude of the responses.
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Means ± SE, 8 Dogs

P • 109 mm Hg

40 80  mm Hg

PCO2 80 mm Hg

FIGURE 3

Increases in aortic pressure and hind-limb perfusion pressure as percentages of maximal response, in dogs with vagi cut. Left: Responses to chemoreceptor stimulation by graded hypoxia; mean systemic arterial Po2 was 114 mm Hg, PCO2 36 mm Hg, and pH 7.35. Right: Responses to chemoreceptor stimulation by graded hypercapnia; mean systemic arterial Po2 was 115 mm Hg, PCO2 38 mm Hg, pH 7.33. SE for Po2 and PCO2 = 1 mm Hg or less.

Responses to Changes in PCO2.—The circulatory effects of changes in CO2 tensions at the carotid chemoreceptors were studied in the same dogs. The blood PCO2 was increased in steps from 39 mm Hg to 81 mm Hg while the Po2 was kept constant, averaging 109 mm Hg. The blood pH was allowed to fluctuate spontaneously with the increasing CO2 tensions. The systemic arterial Po2, PCO2, and pH were normal throughout (means 115 mm Hg, 38 mm Hg, and 7.33, respectively). Figure 2 (bottom) illustrates an example of the vascular responses observed at four levels of PCO2. The stimulus-response curves for the group are shown in Figure 3 (right top and bottom). For each PCO2 studied, the standard errors of the mean did not exceed 1 mm Hg. A small but significant response occurred at a mean PCO2 of 39 mm Hg, even though this was not different from the average systemic PCO2 of the dogs. Since, in the normal range of CO2 tensions, the curve showed no plateau segment, small differences in PCO2 (and pH) in the individual experiments may have been sufficient to cause the vascular changes. With increasing CO2 tensions, there was an almost linear increase in the circulatory responses until a PCO2 of 71 ± 1 mm Hg was reached; then the curves started to flatten. The curves for the changes in aortic pressure and hind-limb perfusion pressure were similar, and the maximal increases in pressure averaged 81 ± 14 and 68 ± 16 mm Hg, respectively, at a CO2 tension of 81 mm Hg. Paired comparison (t-test) of these responses with those to hypoxia showed no statistical difference (P > 0.05).

Dilatation of the saphenous vein also increased progressively with the intensity of the stimulation in the five dogs (Fig. 2). The initial dilator response was seen at CO2 tensions of 50-53 mm Hg in four dogs and at 64

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mm Hg in one. It was maximal with a Pco$_2$ of 61–81 mm Hg.

**Responses to Combined and Separate Changes in Pco$_2$ and pH.** In five of these dogs, the CO$_2$ tensions and pH of the blood were varied separately, and the responses were compared with those to simultaneous changes in the same dogs. Hypercapnia with normal pH (mean 7.38) was obtained by adding bicarbonate to the blood of the reservoir for each step increase in CO$_2$ tension. Responses were studied at three levels of CO$_2$ tension. After Pco$_2$ and pH were returned to normal, the blood pH was decreased by steps, from 7.46 ± 0.02 to 6.87 ± 0.02, by the addition of 0.1N HCl to the blood. The chemoreceptors were stimulated at four levels of pH, while the O$_2$ and CO$_2$ tensions were kept normal (means 106 and 40 mm Hg, respectively). The mean systemic arterial Po$_2$, Pco$_2$, and pH values were 114 mm Hg, 38 mm Hg, and 7.31, respectively.

An example of the responses to simultaneous and separate changes in CO$_2$ tension and pH is shown in Figure 4. The greatest effects were caused by hypercapnia with spontaneous decrease in pH, and the maximal response to this stimulus was used as the point of reference (100%) for constructing the curves in Figure 5. When the pH was kept normal, hypercapnia still caused changes in both aortic pressure and hind-limb perfusion pressure, but the curve was depressed to the right of that with hypercapnic acidosis. With normal pH, the response to the same degree of hypercapnia was decreased by more than 60%. Acidosis with normocapnia was a potent stimulus, and the maximal responses obtained, at a pH of 6.87, were 83% of the maximal responses to hypercapnic acidosis. However, for equivalent changes in pH, the responses to acidosis alone were approximately 50% of those obtained with combined hypercapnic acidosis.

The responses were further compared by plotting, on the same graph, the vascular effects of hypercapnic acidosis ($\Delta$ Pco$_2$ + pH) against the sum of the responses to hypercapnia and acidosis ($\Delta$ Pco$_2$ + $\Delta$ pH) separately (Fig. 6). The levels of CO$_2$ tension and pH to be compared were taken from the hypercapnic acidosis curves. The responses to the same levels of Pco$_2$ and pH separately were
Increases in mean aortic pressure and hind-limb perfusion pressure as percent of maximal response to hypercapnic acidosis in dogs with vagi cut. Mean systemic arterial \( Po_2 \) was 114 mm Hg, \( Pco_2 \) 38 mm Hg, pH 7.31. Left: Responses to chemoreceptor stimulation by graded hypercapnia with spontaneously changing pH and with normal pH. Right: Responses to chemoreceptor stimulation by decreasing pH with normal \( Pco_2 \). SE for \( Pco_2 \) given when > 1 mm Hg.

The responses of both aortic pressure and hind-limb perfusion pressure lay to the left of the line of identity, indicating that simultaneous changes in CO2 tension and pH had a greater effect than the sum of each separately. This potentiation was particularly marked for CO2 tensions between 50 and 71 mm Hg.

Effect of \( Pco_2 \) on Responses to Hypoxia.—In five dogs, the carotid bodies were stimulated with graded hypoxia, at different CO2 tensions and pH in the perfusing blood. Systemic arterial blood gases and pH were normal (\( Po_2 \) 105 mm Hg, \( Pco_2 \) 37 mm Hg, pH 7.36). The first curve was obtained at normal \( Pco_2 \) (38 ± 0.4 mm Hg) and pH (7.37 ± 0.01), the second in the presence of hypocapnic alkalosis (\( Pco_2 \) 21 ± 1 mm Hg, pH 7.53 ± 0.02), and the third with hypercapnic acidosis (\( Pco_2 \) 55 ± 0.4 mm Hg, pH 7.21 ± 0.01). The changes in aortic pressure and hind-limb perfusion pressure were used to plot the curves (Fig. 7). Hypocapnic alkalosis markedly decreased the responses to hypoxia, and the curves were depressed to the left in all experiments. The maximal responses were decreased by more than 50%. The effects of hypercapnic acidosis on the responses to hypoxia were more variable. The first determination on this curve (\( Po_2 \) 106 ± 2 mm Hg) represented the response to hypercapnic acidosis. The responses at all levels of hypoxia were increased, shifting the whole curve to the right. In two of the five dogs, the response to combined hypoxia and hypercapnic acidosis was larger than the sum of the responses to
Changes in aortic pressure and hind-limb perfusion pressure (ΔP) as percent of maximal responses. Comparison of effect of combined changes in Pco₂ and pH (y-axis) with summation of responses to separate changes in Pco₂ and pH (x-axis) at carotid chemoreceptors (data from Fig. 5). Both curves lay to left of line of identity, indicating that potentiation occurs when stimuli are combined.

In the other three, the response to hypoxia was only slightly increased by combined hypercapnic acidosis. Thus, in the range of O₂ and CO₂ tensions studied, potentiation was inconstant; when it was absent, hypercapnia had only a minimal effect on the maximal responses to hypoxia. This is in marked contrast to the important and constant influence of hypocapnic alkalosis. Although fewer levels of Po₂ were used in this group, changes in CO₂ did not alter the threshold of hypoxia to cause vascular effects.

**Discussion**

The overall circulatory effects of carotid chemoreceptor stimulation are greatly modified by the concomitant reflex respiratory changes (3, 6). To study the primary vascular responses to the chemoreflex mechanism, these respiratory changes were prevented by bilateral vagotomy and artificial ventilation. The aortic arch receptors were denervated; to prevent hypertension, the carotid baroreceptors were left intact but their activity was kept constant by maintaining a constant perfusion pressure at the sinuses, thus eliminating each separately.

![Figure 6](http://circres.ahajournals.org/)

**FIGURE 6**

Changes in aortic pressure and hind-limb perfusion pressure (ΔP) as percent of maximal responses. Comparison of effect of combined changes in Pco₂ and pH (y-axis) with summation of responses to separate changes in Pco₂ and pH (x-axis) at carotid chemoreceptors (data from Fig. 5). Both curves lay to left of line of identity, indicating that potentiation occurs when stimuli are combined.

![Figure 7](http://circres.ahajournals.org/)

**FIGURE 7**

Effect of Pco₂ on vascular responses to graded hypoxic stimulation of carotid chemoreceptors in dogs with vagi cut. Mean systemic arterial Po₂ was 105 mm Hg, Pco₂ 37 mm Hg, 7.36. Left: Increases in mean aortic pressure in response to hypoxia at three levels of Pco₂. Right: Increases in hind-limb perfusion pressure with same stimuli. Varying Pco₂ changed slopes of curves, but threshold Po₂ for vascular effects was not affected. SE for Po₂ given when > 1 mm Hg.

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buffering of the vascular responses by the baroreflex mechanism. At most, increases in pressure of 1-3 mm Hg in the sinus area during stimulation of the chemoreceptors may have slightly diminished the vasoconstrictor responses. In previous experiments, this technique of selective perfusion of the carotid bodies was evaluated, and the circulatory changes after stimulation with hypoxic or hypercapnic blood were shown to be due solely to activation of the chemoreceptors, since they were abolished by blockade of the carotid sinus nerves (5).

Complete isolation of the carotid bifurcations from the systemic circulation was mandatory to avoid mixing of the blood used to stimulate the chemoreceptors with arterial blood from small patent branches. This was not possible in all dogs without damaging the innervation or the venous drainage of the carotid bodies. In such cases, the chemoreceptors were also unresponsive to cyanide injection in the input cannulas, and the experiments were terminated. The large difference between the pressure in the bifurcations and that in the systemic circulation on arrest of the perfusing pump (more than 160 mm Hg in all cases) was a good index of adequate isolation. It was thus possible to vary precisely the $O_2$ and $CO_2$ tensions and pH at the carotid bodies, while the systemic arterial gases and pH were kept constant and normal. Accurate adjustments of the gas tensions and pH in the blood at the chosen levels could be achieved repeatedly from one experiment to the other, and standard errors of the mean of less than 1 mm Hg or 0.02 pH unit were the rule in most curves. The hematocrit was not taken into account since it appears to have no influence on the activity of the chemoreflex mechanism (9, 10).

During perfusion of the bifurcations with blood from the extracorporeal circuit, the venous drainage from the carotid bodies was added to the blood volume of the dog. The amount of blood so transferred was not measured, but it must have been minimal since the level of blood in the reservoir remained almost constant during the perfusion and the arterial blood pressure was the same before and after the stimulation period. Under these conditions, the circulatory reflex responses to different degrees of stimulation of the carotid chemoreceptors could be quantitatively analyzed and stimulus-response curves could be derived.

The results of these experiments indicate that independent changes in $P_{O_2}$, $P_{CO_2}$, or pH at the carotid bodies cause circulatory responses through the chemoreflex mechanism and that their magnitude is proportional to the intensity of the stimulation. Redistribution of blood flow in the carotid body occurs during hypoxia and hypercapnia, and this may contribute to activation of the chemoreceptors (11, 12). Perfusion of the carotid bodies in vivo does not permit one to exclude completely such a local vascular effect, even though there is no relationship between chemoreceptor discharge and total carotid body blood flow, according to Biscoe and collaborators (9). Excitation of the carotid chemoreceptors can also be induced by increased sympathetic nerve activity to the carotid body, but this effect is small (13), and in the present experiments the cervical sympathetic nerves were cut.

The increases in aortic pressure and in perfusion pressure of the isolated hind limb were similar over the whole range of stimulations examined. This supports the conclusion of others that, in the vagotomized and artificially ventilated dog, the changes in arterial pressure caused by the chemoreflex mechanism reflect mainly the increase in peripheral vascular resistance mediated by the sympathetic nervous system (3, 6). This increase in sympathetic activity is not uniformly distributed. Under the same conditions, the carotid chemoreflex has been found to cause small decreases in heart rate and ventricular contractility, and no increase in nerve discharge was obtained from recordings of cardiac sympathetic fibers (3, 14, 15). By increasing the venous return to the heart, the venoconstriction in the splanchnic area might help to maintain or slightly increase the cardiac output (3, 5). In previous experi-
ments, it was shown that the cutaneous veins respond in an opposite manner to the resistance and splanchnic capacitance vessels by dilating upon stimulation of the chemoreceptors when venomotor tone is present. This reflex venodilatation was most likely due to the withdrawal of adrenergic activity, although the participation of a sympathetic dilator system mediated by an unknown substance could not be completely excluded (5). As demonstrated in the present experiments, this response is also proportional to the degree of stimulation of the chemoreceptors. Similarly, the cutaneous resistance vessels dilate in response to the chemoreflex (16). The role of this reflex dilator response of the cutaneous vessels is not known. The cutaneous component of the capacitance system is not under the control of the baroreceptors of the high-pressure and low-pressure systems (17,18), but it participates in the regulation of body temperature (8). Eyzaguirre and Lewin (19) reported that the chemoreceptors of the cat could be activated by increases in temperature in vitro. This observation might indicate a relationship between the chemoreceptors and the cutaneous vessels in the regulation of body temperature.

It has been established that the number of impulses in the carotid sinus nerve increases as the Po2 is decreased below 200 mm Hg, but it is not until Po2 has reached 80-100 mm Hg that a sharp increase in neural discharges is obtained (20, 21). Most of the respiratory reflex responses have been correlated with the O2 content of the inspired gas or with the alveolar Po2; reflex effects were observed between 80 and 100 mm Hg (22-24). The arterial O2 tension at the carotid bodies was not measured and was probably slightly lower. In the present experiments, circulatory reflex effects appeared at Po2 between 70 and 80 mm Hg. The vascular responses are thus closely related to the Po2 at which there is a rapid increase in chemoreceptor activity. Even though the nerve impulses increase when the Po2 is decreased from 200 to 100 mm Hg, the rate of increase is small and probably does not reach the threshold necessary to cause reflex responses. At O2 tensions below 80 mm Hg, the increase in nerve impulses is nearly linear (20, 21), as were the changes in hemodynamic pressures in these experiments, and the curves do not indicate any tendency toward a plateau over the ranges of Po2 studied.

Hypercapnic acidosis also results in increased electrical activity of the sinus nerve and in reflex ventilatory responses, and these responses are more or less linearly related to the Pco2 between 25 and 65 mm Hg (2, 21, 25). The levels of Pco2 at which the increase in nerve activity reached its maximum were 80 and 110 mm Hg in two studies (20, 21). Over the range of CO2 tensions of 40-80 mm Hg, vascular reflex effects similar to those with hypoxia were obtained. However, the tendency toward flattening of the curves above 70 mm Hg was not seen with hypoxia. This is in contrast to the results of Daly and collaborators (4, 26), who found little or no vascular changes in response to chemoreceptor stimulation with hypercapnia. Although the high level of O2 used in their experiments may explain these negative findings (11), other important factors could have been involved. Hornbein and Roos (27) and Gray (28) reported that changes in CO2 are ineffective in activating the chemoreceptors if not accompanied by decreases in pH. This has been challenged by Biscoe and colleagues (21), who showed, using the single-fiber recording technique, that hypercapnia can cause an increase in nerve impulses when pH is maintained normal, even though the response is much attenuated. The peripheral vascular changes observed in the present study during hypercapnia with both normal and decreased pH are in agreement with the latter. That redistribution of blood flow within the carotid body might be at least partly cause for the response cannot be discarded (11). In addition, when bicarbonate was added to the blood to correct pH, the resulting changes in osmolarity might have altered carotid body blood flow, but there is no evidence for or against this possibility. However, using the superfused carotid body in vitro, Eyzaguirre and Koyano (25) also found that high CO2
with normal pH causes an increase in chemoreceptor discharge.

The importance of pH as a chemoreceptor activator has been well established (21, 25, 27) and is further substantiated by the circulatory reflex effects obtained in this study. According to Gray (28), changes in pH either by increasing CO₂ tension or by decreasing bicarbonate concentration result in the same increase in activity, as measured from whole-nerve preparations. The present results, on the contrary, suggest that the effect of acidosis is potentiated by combined hypercapnia. The relationship between the actions of CO₂ and pH on the receptor cells of the carotid body is still unclear. It has been suggested that pH acts indirectly through its effect on a presynaptic transmitter substance, probably acetylcholine (29). On the other hand, when pH is kept normal, the effect of hypercapnia may be mediated by changes in intracellular pH rather than by a direct action of the CO₂, since it diffuses into the cells more rapidly than the bicarbonate ion (27). This explanation would also account for the faster effect of hypercapnic acidosis as compared to acidosis obtained by fixed acids (28). In this case, total CO₂ content of the perfusate would be an important determinant of the changes in intracellular pH and could explain the discrepancy between the results of the present study and those of Gray (28), who used a buffered saline solution. At any rate, it is not claimed that CO₂ has a direct effect on the receptors but rather that hypercapnic blood with normal pH causes reflex vascular responses and that the effect of hypercapnia is markedly affected by the pH.

Similarly, the responses to hypoxia were largely modified by the CO₂ tension of the perfusing blood. Potentiation of the effects of hypoxia by hypercapnia has been reported (26), but this was not found consistently in the present experiments. However, hypocapnic alkalosis always decreased the responses to hypoxia markedly. Thus, if the stimulus-response curve to hypoxia at high PO₂ is compared with that at low rather than normal PO₂, it may be concluded that potentiation has occurred. It has been shown by Hornbein (20) that both the threshold and the slope of the stimulus-response curve to hypoxia are changed by varying CO₂ tension. The present results convincingly demonstrated the influence of PO₂ on the slope of the curves to hypoxia, but the PO₂ threshold for the vascular effects did not appear to be modified to any marked degree. Since changes in pH alone were found to have the same effect on the responses to hypoxia (27), this may be the underlying cause of at least part of the changes attributed to CO₂. This emphasizes the prime and determinant role of the pH in the excitation process of the chemoreceptors, and the difference between the findings of Daly and colleagues (4, 26) and the results of the present experiments could be explained on this basis. Recently, it has been suggested that the rate of change in pH may have a more important influence on the receptor cells than its absolute value (30). If the chemoreceptor cells were sensitive to the rate of changes of the various stimuli, the vascular responses observed in the present experiments might have been exaggerated by the almost square-wave type of stimulation used. However, the responses were well sustained during the period of stimulation (2-4 minutes).

Although the same pattern of responses in aortic pressure and hind-limb and saphenous vein perfusion pressures was observed in all experiments, the magnitude of the responses was strikingly variable from one dog to another. Whether this was due to a difference in the circulatory influence of the carotid bodies in each animal or to partial damage during the surgical preparation cannot be stated at this time. Most often, potent reflex circulatory responses could be evoked by carotid chemoreceptor stimulation with hypoxia, hypercapnia, or acidosis. Based on the stimulus-response curves obtained, the chemoreflex mechanism probably has only a limited influence on the circulation with the normal fluctuations in blood gases and pH in the anesthetized dog. However, even though the reflex may have been depressed by general anesthesia, vascular effects were readily ob-
served with small increases in CO₂ tension above normal, as contrasted with the much larger decreases in PO₂ necessary to cause similar changes. In the cat, the chemoreflex has been assigned a role in the control of blood pressure during sleep; although the exact mechanism remains unknown, changes in carotid body blood flow, hypoxia, or a moderate degree of hypercapnia have been suggested as possible causes (31). In hypoxia, hypercapnia, or acidosis, the chemoreceptors, through their potent vasoconstrictor effect, become a major regulator of the circulation and oppose the vasodilatation caused by the lung inflation reflex (32) and by the local effects on the blood vessels (33).

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References

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