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Regulation of Blood Flow to Respiratory Muscles during Hypoxia and Hypercapnia (41039)[1]

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Abstract. The effect of systemic hypoxia or hypercapnia on regional blood flow was measured in respiratory muscles, limb muscles, and kidneys of rabbits by using radioactive microspheres. Blood flow to both the diaphragm and the intercostal muscles was increased in a direct relationship with the severity of the hypoxia or hypercapnia in spontaneously breathing animals. Blood flow to the kidneys decreased while blood flow to limb muscle remained unchanged. During mechanical ventilation, following paralysis with Flaxedil, blood flow to the respiratory muscles was about 10% of that seen during quiet breathing and was unchanged by hypoxia or hypercapnia. Renal flow decreased as in the nonparalyzed animals while flow to limb muscle remained unchanged during the hypoxic and hypercapnic episodes. It is concluded that the major factor involved in regulating blood flow to the respiratory muscles is their level of activity.

Blood flow to the respiratory muscles, and in particular to the diaphragm, increases during hypoxic or hypercapnic (1–5) conditions. At least three possible mechanisms may be considered to be involved in this response. First, the increased respiratory activity resulting from these conditions increases the work of these muscles. Blood flow to the respiratory muscles has been shown to increase with respiratory work load (5–7). Second, the direct local effects of low O_2 or high CO_2 on the vascular smooth muscle of the respiratory muscles may produce a vasodilation in these beds (8). Last, stimulation of chemoreceptor reflexes may produce reflex vasodilation in respiratory muscle (1). The present studies were undertaken to investigate the relative role these mechanisms may serve in increasing blood flow to the respiratory muscles in conditions of hypercapnia or hypoxia. The results suggest that the major factor involved is the level of activity in the respiratory muscles.

Methods. A total of 42 New Zealand white rabbits (3–4 kg) were anesthetized with sodium pentobarbital (20–30 mg/kg) given into the marginal ear vein. Additional anesthetic was given as necessary during the experiment. The trachea was cannulated. In one group of 19 animals, the experimental protocol was to increase systemic PCO_2 from the control level (about 30 Torr) while keeping systemic PO_2 at the control level (about 100 Torr). Nine of these animals were paralyzed with gallamine triethiodide (Flaxedil, 1–2 mg/kg) and ventilated with a Harvard small animal respirator. The respirator rate was set at 35–40 breaths/min. Tidal volume of the respirator was adjusted to produce control PO_2, PCO_2, and pH levels. The remaining 10 animals in this group breathed spontaneously from an anesthesia bag containing a gas mixture of air, O_2, and CO_2. One-way valves directed gas flow from the bag during inspiration and to the atmosphere during expiration. These valves offered little resistance to gas flow and therefore did not significantly increase inspiratory or expiratory work. The respiratory mixture was adjusted through the use of mixing valves so that systemic PO_2 and PCO_2 could be varied or kept constant depending on the experimental variable. In these animals the CO_2 in the gas mixture was increased from control until systemic blood PCO_2 reached levels of about 40, 65, and 75 Torr. The O_2 in the mixture was adjusted so that systemic PO_2 was kept at the normal level. The animal was maintained on a given gas mix-

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ture until two readings of the blood gases taken at 5 min intervals were identical. At this time regional flow determinations were made. Blood pressure, breathing rate, and heart rate were also recorded. After obtaining these data the animal was allowed to return to control conditions for about 30 min and was then subjected to a different level of CO_2 and the above procedure repeated. The CO_2 levels were introduced in a random order. The 9 animals in this group which were paralyzed and mechanically ventilated were subjected to a similar protocol. Regional flow data were only obtained at control and high CO_2 levels in these animals.

In a second group of 23 animals (15 control, 8 paralyzed) the effect of hypoxic conditions was studied. In these animals O_2 in the gas mixture was reduced from control levels (PO_2 = 100 Torr) to levels which produced systemic PO_2 values of about 45 and 25 Torr while PCO_2 was kept at a normal level. Following each hypoxic episode the animal was returned to normoxia until recovery of control conditions appeared complete. Following recovery a different level of hypoxia was introduced. Seven of the control animals were excluded from the severe hypoxia data because they failed to recover satisfactorily from this episode.

Eight animals in this group were paralyzed with Flaxedil and ventilated artificially as described above. Data were only obtained during the control and severe hypoxic conditions in these animals.

In order to abolish cardiopulmonary and aortic reflexes both vagi and aortic nerves were cut at a midcervical level in all animals. The changes in respiratory rate in these animals to hypoxia are similar to those observed in earlier experiments where these nerves were left intact. A polyvinyl catheter connected to a pressure gage was advanced down the right carotid artery into the left ventricle. The position of this catheter was confirmed by observing the contour of the pressure pulse and by inspection at autopsy. This catheter was used for the injection of microspheres. A second catheter was advanced through the femoral artery into the abdominal aorta. This catheter was used for the collection of blood samples. A third catheter in the opposite femoral artery was used to monitor blood pressure. Heart rate was recorded from a cardiotachometer which was triggered by the pressure tracing. Pressure recordings were made with Statham strain gages recording on a Beckman recorder. Breathing was recorded from a strain gage around the thoracic cavity.

In all animals regional blood flow determinations were made by using microspheres (3M Co., New England Nuclear) with a mean diameter of 15 μM. The microspheres were labeled with one of the following isotopes: ^{125}I, ^{109}Co, ^{57}Co, ^{46}Sc, or ^{85}Sr. Approximately 400,000 microspheres suspended in 1 ml 63% sucrose were injected as a bolus into the left ventricle. The catheter was flushed with 2 ml saline. A 3-ml blood sample was simultaneously withdrawn from the abdominal aorta by a Harvard withdrawal pump at the rate of 2 ml/min. This sample comprised an integrated arterial flow standard and was used for calculating regional flows.

Following completion of the experimental procedures the animals were sacrificed and the entire diaphragm (4–5 g), the total obtainable intercostal muscles (10–15 g), a 10 to 15-g sample of forelimb muscle and both kidneys (2–3 g each) were removed, weighed, and assayed for radioactivity. Each tissue was divided into 2- to 5-g samples and placed into wide-mouth counting vials. The vials were placed in a well-type three-channel Nuclear Chicago automatic gamma counter (Mdl 1185) and counted for 10 min or until 400,000 counts were obtained. No effort was made to separate the internal and external intercostal muscles. The lungs were also removed and assayed to determine the extent of arterial-venous shunting of microspheres.

A standard vial of each isotope used allowed determination of the crossover between the various channels and the number of counts per minute per sphere. This information, together with the counts in each sample and the dilution of each sample were used to determine blood flow to each organ using computations previously described (9, 10). All computations were performed on a Sperry Univac 1110 computer.

In all cases the tissue samples assayed trapped more than 400 spheres, a minimum number necessary to achieve 95% confidence that the flow estimates are within 10% of the true value (11). Activity in the lungs was less than 10% of the total activity and was not altered by the experimental procedures. Right and left kidney flow differed by less than 15% indicating homogenous mixing of the microspheres in the blood stream.

Statistical comparisons of the data were made by the standard paired *t* test. The 95% level of confidence was selected for significance.

Results. The results of all experiments are summarized in Tables I and II. In the spontaneously breathing animals blood flow to the respiratory muscles increased as the severity of either hypoxia or hypercapnia was increased. This increase was somewhat more pronounced in the diaphragm than in the intercostal muscles. Flow to the diaphragm increased about threefold during the most severe state of either hypoxia or hypercapnia. Flow to the intercostal muscles increases two- to threefold. Flow to nonrespiratory muscle showed little change while renal flow decreased as the severity of the hypoxia or hypercapnia increased. Arterial blood pressure was unchanged by either hypoxia or hypercapnia, therefore, changes in flow reflect the changes in regional vascular resistance that occurred during the hypoxic or hypercapnic episode. The decreased renal flow is the expected response to excitation of the chemoreflexes during hypoxia or hypercapnia. Blood flow to nonrespiratory skeletal muscle would also be expected to decrease during chemoreceptor stimulation (12, 13). The fact that this did not occur may result from the inability to accurately measure decreases in flow from the low base level of control flow in these inactive muscles or may result from an offsetting vasodilation which occurred in response to the change in the local levels of O_2 and CO_2.

Blood flow to the diaphragm during normal quiet breathing averaged 45–50 ml/min/100 g in our experiments. This is in agreement with other reported values for rabbits (5, 6). Paralyzing the animal with

TABLE I. THE EFFECT OF GRADED HYPERCAPNIA ON REGIONAL BLOOD FLOW

		Organ flow ±SD (ml/min/100 g)					
Organ	pH	7.45 ± 0.05	7.31 ± 0.09	7.18 ± 0.09	7.08 ± 0.06	7.39 ± 0.01	7.14 ± 0.10
	PO_2 (Torr)	106 ± 18	111 ± 18	114 ± 9	105 ± 11	119 ± 24	128 ± 23
	PCO_2 (Torr)	30 ± 3	42 ± 4	65 ± 8	78 ± 11	29 ± 7	60 ± 10
		Nonparalyzed group $N = 10$			Paralyzed group $N = 9$		
Diaphragm		53 ± 20	94 ± 32*	132 ± 45*	151 ± 71*	3.9 ± 2.3	5.3 ± 3.1
Intercostal muscle		3.0 ± 0.6	6.8 ± 4.9*	6.5 ± 3.8*	10.2 ± 7*	1.9 ± 0.8	2.6 ± 0.8
Forelimb muscle		2.3 ± 0.6	2.7 ± 1.1	2.2 ± 0.8	3.7 ± 1.9	2.3 ± 0.5	2.2 ± 0.5
Kidney		353 ± 94	236 ± 160*	180 ± 66*	159 ± 67*	255 ± 122	139 ± 85*
Respiratory rate (breaths/min)		51 ± 10	65 ± 13*	61 ± 8*	63 ± 10*		
Systemic art. press. (mm Hg)		87 ± 12	85 ± 17	91 ± 10	91 ± 15	74 ± 16	76 ± 14
Heart rate (beats/min)		282 ± 32	270 ± 38	237 ± 43*	236 ± 26*	282 ± 10	232 ± 25*

* $P < 0.05$ value different from value during control conditions.

TABLE II. THE EFFECT OF GRADED HYPOXIA ON REGIONAL BLOOD FLOW

		Organ flow ± SD (ml/min/100 g)				
	pH	7.41 ± 0.09	7.39 ± 0.09	7.40 ± 0.06	7.36 ± 0.04	7.36 ± 0.06
Organ	PO_2	105 ± 28	44 ± 4	27 ± 2	115 ± 17	25 ± 4
	PCO_2	28 ± 4	28 ± 5	31 ± 4	33 ± 5	34 ± 6
		Nonparalyzed group N = 15		N = 8	Paralyzed group N = 8	
Diaphragm		44 ± 25	89 ± 45*	154 ± 69*	4.5 ± 2.1	4.3 ± 2.1
Intercostal		4.1 ± 2.4	7.3 ± 3.9	8.4 ± 3.6	2.9 ± 2.4	2.0 ± 1.5
Gastrocnemius		2.1 ± 0.8	3.4 ± 1.4	3.9 ± 1.4	2.7 ± 1.8	2.6 ± 2.0
Kidney		405 ± 139	184 ± 112*	199 ± 123*	305 ± 96	113 ± 102*
Resp. rate		50 ± 13	64 ± 10*	61 ± 11*		
Systemic art. press. (mm Hg)		83 ± 16	79 ± 14	92 ± 11	88 ± 10	90 ± 11

* $P < 0.05$ value different from value during control conditions.

Flaxedil and maintaining ventilation with a respirator reduced diaphragmatic flow by roughly 90%. Part of this reduction is due to a somewhat lower level of systemic pressure in these animals but most is due to the increased level of resistance in the nonworking diaphragm. Neither hypoxia nor hypercapnia caused an increase in flow to the noncontracting respiratory muscles in the paralyzed animals. Renal blood flow, however, was reduced by both hypoxia and hypercapnia in the paralyzed animals as it was in the nonparalyzed group. This suggests that the chemoreflexes are equally active in both groups.

As expected respiratory rate was increased by both hypoxia and hypercapnia. The maximum rate was reached at intermediate levels of PO_2 and PCO_2. The respiratory movements were also observed to increase in depth as PO_2 decreased and PCO_2 increased. Measurement of these changes in depth was dependent on the placement of the strain gage around the thorax and therefore not quantifiable.

Discussion. The present studies have shown that progressive increments in the severity of either hypoxia or hypercapnia causes progressive increases in blood flow to contracting respiratory muscles. This effect is somewhat more pronounced in the diaphragm than in the intercostal muscles but in either muscle, flow may increase two- to threefold over the value seen during quiet breathing. A similar increase is not observed in the noncontracting respiratory muscles. In these muscles resting flow is about 10% of that seen during quiet breathing. These results suggest that the major factor involved in increasing flow to the respiratory muscles during either hypoxia or hypoxemia is the increased level of activity in these muscles. In our experiments we only measured breathing rate and therefore are unable to relate blood flow and respiratory work. Mognoni *et al.* (6) observed a doubling of diaphragmatic flow when ventilation was tripled in rabbits during CO_2 hyperventilation. Axeń and Janson (5) reported that lung ventilation in rabbits increased 53% in response to breathing 2% CO_2, 98% O_2. Diaphragmatic flow increased 28%. Robertson *et al.* (7) observed in dogs that flow to the diaphragm increased linearly with increased respiratory work caused by CO_2 hyperventilation but increased exponentially as work was increased by increasing inspiratory resistance. Rochester (2) on the other hand, only observed a 25% increase in diaphragm flow when minute ventilation was doubled in dogs breathing a hypoxic–hypercapnic gas mixture.

The resting values for blood flow to the contracting diaphragm that we have observed in rabbits (45–55 ml/min/100 g) are in good agreement with those reported by others (5, 6). They are in general higher than those seen in the dog (1–4). Magnoni *et al.* (6) reported resting a flow value of 12.8 ml/min/100 g to the intercostal muscles. Our values are roughly one-third of

their value. We made no effort to separate the inspiratory from the expiratory intercostals as Mognoni did which may partially account for the discrepancy.

In the noncontracting paralyzed diaphragm we observed blood flows comparable to those seen in nonrespiratory skeletal muscle (4–5 ml/min/100 g). These values are only about 10% of that seen during normal quiet breathing. Mognoni *et al.* (6) observed basal flow levels to the diaphragm of 16 ml/min/100 g after cutting the phrenic nerves. It is not clear in their report whether the sympathetic innervation of the diaphragm was also destroyed. This will depend upon the level of phrenic nerve transection (14). In our experiments we would expect the sympathetic innervation of the diaphragm to be intact in the paralyzed animals. This may explain the difference between the basal flows to the noncontracting diaphragm observed in these studies. Robertson *et al.* (4) observed in dogs that flow to the noncontracting diaphragm was 4 ml/min/100 g while during quiet breathing this flow increased to 9 ml/min/100 g.

In our experiments blood flow to the noncontracting diaphragm or intercostal muscles was not increased by systemic hypoxia or hypercapnia. This suggests that the flow response observed in these muscles in the spontaneously breathing animals cannot result from chemoreflex activation or from local dilating effects of the low systemic O_2 or high systemic CO_2.

In conclusion it appears that the increased flow to the respiratory muscles in response to systemic hypoxia or hypercapnia is closely related to the increased activity of these muscles and does not involve vascular reflexes. Severe systemic hypoxia or hypercapnia also does not appear to directly produce sufficient local vasodilation to account for the increased flow to the respiratory muscles. This, however, does not exclude the possibility that tissue levels of O_2 and CO_2 in the working muscles may cause marked local vasodilation.

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