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Neural and Metabolic Control of Blood Flow to Respiratory Muscles of Rabbits[1] (41202)

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Abstract. Regional blood flow was measured in respiratory muscles, limb muscles, and kidneys of rabbits anesthetized with sodium pentobarbital. Flow measurements were made by the use of radioactive microspheres. The responses to graded aortic nerve (AN) stimulation on regional blood flows at different levels of inspiratory resistance were determined. As the inspiratory load was increased blood flow to respiratory muscles increased due to a decreased vascular resistance. Blood flow to the limb muscle and kidneys decreased. Stimulation of the AN increased blood flow and decreased vascular resistance in the respiratory muscles at all levels of respiratory activity studied. The stimulus-response curves to AN stimulation were similar in paralyzed animals, animals breathing quietly, and in animals breathing against an inspiratory resistance sufficient to produce a peak inspiratory pressure of 15 mm Hg. These results suggest that sympathetic control of the blood flow to respiratory muscles may be important during normal breathing and during labored breathing where metabolic vasodilation exists.

Although sympathetic control of vascular resistance in limb skeletal muscle has been extensively studied no assessment of the sympathetic regulation of blood flow to respiratory muscles appears to have been made. Rochester and Briscoe (1) concluded that the skeletal muscle of the diaphragm more closely resembles cardiac than limb skeletal muscle in its perfusion and metabolic behavior. Based on this assumption we would expect the vascular bed of the diaphragm to be predominantly regulated by local metabolic factors and minimally affected by sympathetic vasoconstrictor activity. Several studies have shown a close relationship between respiratory activity and blood flow to the respiratory muscles (2–8).

In the present study we have investigated the effect of the depressor reflexes in the aortic nerve (AN) on vascular resistance in the respiratory muscles at different levels of respiratory activity. Our assumption is that the AN depressor reflexes inhibit sympathetic vasoconstrictor activity. The results suggest that sympathetic vasoconstrictor regulation of blood flow to the respiratory muscles is important over a wide range of respiratory activity.

Methods. A total of 35 New Zealand white rabbits (3–4 kg) were anesthetized with sodium pentobarbital (20–30 mg/kg) given into a marginal ear vein. Additional anesthetic was given as necessary during the experiment. The trachea was cannulated and all animals, except the paralyzed group (N = 10), breathed from a spirometer containing air enriched with O_2. The O_2 in the mixture was adjusted so that blood PO_2 was about 100 Torr during the experimental procedures. Blood PCO_2 was about 30 Torr and pH between 7.35 and 7.42 during the experiments. One-way valves directed gas flow from the spirometer into the trachea during inspiration and directed the expired gases to the atmosphere. These valves offered little resistance to airflow. A screw valve between the spirometer and the inspiratory valve provided a means of increasing the inspiratory resistance without changing the resistance to gas flow during expiration. Thus, inspiratory load could be varied while expiratory load remained constant. Intratracheal pressure was measured in the tracheal cannula. Peak inspiratory pressure (PIP) was used as an index of inspiratory load. During quiet breathing this

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averaged 2 mm Hg and was increased by changing the airway resistance in the inspiratory circuit during the experiments. In 10 of these animals only the effects of increasing inspiratory resistance on regional blood flow was studied. In seven of these animals both carotid arteries were occluded and both aortic nerves (AN) and vagus nerves were cut in the midcervical region. This procedure was conducted to minimize changes in regional blood flow which might result from reflex activity triggered by the increased breathing effort caused by inspiratory loading. Inspiratory resistance was set such that PIP were about 2, 13, 17, and 23 mm Hg. Each PIP setting was maintained (about 10 min) until steady-state conditions in blood pressure, breathing rate, and blood gases were reached. Determination of regional flows were then made. Inspiratory resistance was decreased to control level and a 15-min recovery period allowed before subjecting the animal to the next inspiratory load. The inspiratory resistance levels were selected at random. In three animals the vagi were left intact and one carotid artery left patent. The other carotid artery was occluded by the ventricular catheter.

In the remaining 25 animals the regional flow responses to AN stimulation at different levels of inspiratory load were determined. As far as possible the experimental conditions in these animals were the same as those described above. Both vagi and AN were cut and both carotid arteries were occluded. The central ends of the AN were drawn into silver ring electrodes for stimulation. Stimulus parameters were set at 1.0 msec and stimulus voltage was adjusted (1–6 V) to cause a maximum depressor response at a frequency of 100 Hz. The stimulus frequencies of 10, 20, and 100 Hz were used in random order and regional flow determinations were made under steady-state conditions at each frequency setting. A recovery period of 10–15 min was allowed between stimulation periods. Flow responses to the AN stimulation series were determined at PIP levels of about 0, 2, and 15 mm Hg. Higher levels of inspiratory resistance were not used because stable conditions could not always be maintained for the time necessary for the AN stimulation series.

Ten of these animals were paralyzed with gallamine triethiodide (Flaxedil, 1–2 mg/kg) and ventilated mechanically with a Harvard small animal respirator (PIP = 0). The remaining 15 animals breathed spontaneously; eight against minimal inspiratory resistance (PIP = 2 mm Hg); and seven against a high inspiratory resistance (PIP = 15 mm Hg).

In all animals a polyvinyl catheter connected to a pressure gauge 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 femoral pressure tracing. Pressure recordings were made with Statham strain gauges recording on a Beckman polygraph. Breathing rate was recorded with a pressure gage connected to a needle in the tracheal cannula.

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}Cd, ^{57}Co, ^{46}Sc, or ^{85}Sr. Approximately 400,000 microspheres of a species were suspended in 1 ml 63% sucrose and rapidly injected 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. Four species of microspheres were injected into each animal.

Following completion of the experimental procedures the animals were sacrificed

and the entire diaphragm (4–5 g), the total obtainable intercostal muscle (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. The lungs were also removed and assayed to assess the extent of arteriovenous shunting of microspheres. Each tissue was divided into 2- to 5-g samples and placed in 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.

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). All computations were performed on a Sperry Univac 1110 computer.

In all cases the tissue samples assayed trapped more than 400 spheres, the minimum number necessary to achieve 95% confidence that the flow estimates are within 10% of the true value (10). Activity in the lungs never exceeded 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 within groups were made by the standard one-way analysis of variance. Statistical comparisons between groups were made by the unpaired t test (11). The 95% level of confidence was selected for significance.

Results. Figure 1 and Table I summarize the effects of increasing inspiratory resistance on regional blood flow in seven animals. Flow to the respiratory muscles increased as inspiratory resistance increased. With the maximum inspiratory resistance used, diaphragmatic flow increased threefold and intercostal flow doubled. Renal flow and flow to nonrespiratory skeletal muscle were reduced ($P < 0.05$) by about 44 and 33%, respectively, at this level of inspiratory resistance. Systemic blood pressure was essentially unchanged by increasing inspiratory resistance. Thus, the changes in regional blood flows reflect changes in regional vascular resistance. It seems certain from Fig. 1 that further vasodilation in the respiratory muscles would have occurred if the inspiratory load had been further increased. However, PIP values greater than 25 mm Hg resulted in decreased systemic pressure, decreased PO_2, and increased PCO_2 and pulmonary edema if maintained for the period necessary to obtain stable conditions. We therefore limited inspiratory load to this value. In three animals not included in Table I, the AN and vagi were left intact and one carotid artery was left patent. The effects of increasing inspiratory resistance were studied as outlined above. Raising PIP from the resting level of 2 to 20–25 mm Hg caused flow to the diaphragm to increase from an average of 45 to 83 ml/(min × 100 g). Intercostal flow changed from 3.2 to 4.9 (ml/(min × 100 g) over this same range of inspiratory load.

The data presented in Table II and Fig. 2 summarize the effects of graded AN stimulation on regional flow and resistance at different levels of respiratory activity. Blood flow to the respiratory muscles

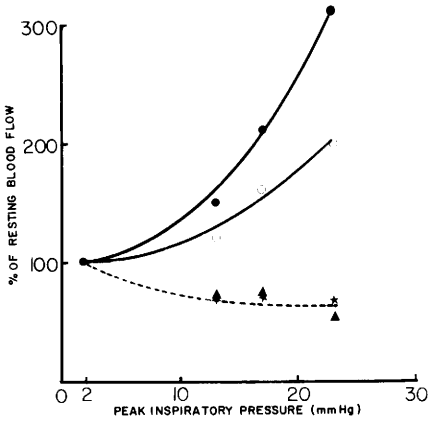

FIG. 1. Effect of increasing inspiratory resistance on regional blood flow. Change in flow expressed relative to flow values observed during quiet breathing with no added inspiratory resistance (PIP = 2 mm Hg). Flow curves for diaphragm: closed circles; intercostal muscle: open circles; limb muscle: stars; and kidney: triangles.

TABLE I. REGIONAL FLOW AND RESISTANCE VALUES AT DIFFERENT LEVELS OF INSPIRATORY LOAD. $N = 7$

Peak inspiratory pressure (mm Hg)	2.0 ± 0.8		13.3 ± 1.0		17.4 ± 0.5		23.3 ± 1.6	
Organ	Q ± SD	R ± SD	Q ± SD	R ± SD	Q ± SD	R ± SD	Q ± SD	R ± SD
Diaphragm	34 ± 6	3.1 ± 0.8	52 ± 6*	2.0 ± 0.3*	72 ± 12*	1.5 ± 0.2*	106 ± 28*	1.1 ± 0.3*
Intercostal	3.7 ± 0.7	29 ± 3	4.4 ± 0.9*	24 ± 5*	6.1 ± 0.7*	18 ± 2*	7.4 ± 1.6*	15 ± 4*
Limb Muscle	3.9 ± 0.6	27 ± 6	2.9 ± 0.6	36 ± 7	2.9 ± 0.9	39 ± 11	2.6 ± 0.8*	45 ± 15*
Kidney	304 ± 78	0.35 ± 0.1	231 ± 67	0.48 ± 0.1	235 ± 52	0.47 ± 0.1	171 ± 70*	0.74 ± 0.3*
Respiratory rate (breaths/min)	34 ± 8		41 ± 3		38 ± 7		37 ± 9	
pH	7.45 ± 0.07		7.42 ± 0.02		7.49 ± 0.1		7.44 ± 0.1	
PO_2 (Torr)	148 ± 19		122 ± 34		129 ± 37		114 ± 36	
PCO_2 (Torr)	27 ± 3		26 ± 2		25 ± 2		28 ± 5	
Heart rate (beats/min)	326 ± 19		328 ± 18		293 ± 34		300 ± 23	
Blood pressure (mm Hg)	103 ± 9		101 ± 2		106 ± 6		110 ± 12	

* Values different from control values ($P < 0.05$). Q = flow [ml/(min × 100 g)]. R = resistance [mm Hg/ml/(min × 100 g)].

TABLE II. REGIONAL FLOW AND RESISTANCE VALUES DURING STIMULATION OF AORTIC NERVES AT DIFFERENT INSPIRATORY LOADS

Aortic nerve stimulation (Hz)			0		10		20		100	
Organ	*N*	Peak inspiratory pressure (mm Hg)	*Q*	*R*	*Q*	*R*	*Q*	*R*	*Q*	*R*
Diaphragm	10	0	2.3 ± 0.5	78 ± 25	3.1 ± 0.1	44 ± 21	3.7 ± 1.2	28 ± 12	5.3 ± 1.7	16 ± 6
	8	2	29 ± 7	5.1 ± 1.1	40 ± 10	2.5 ± 0.5	49 ± 8	1.8 ± 0.6	51 ± 11	1.4 ± 0.5
	7	15	56 ± 4	2.3 ± 0.3	54 ± 9*	1.7 ± 0.2	67 ± 10	1.2 ± 0.2	75 ± 6	0.8 ± 0.2
Intercostal muscle	10	0	1.6 ± 0.5	113 ± 40	1.9 ± 0.8*	78 ± 40	2.3 ± 0.8	50 ± 26	4.2 ± 1.7	23 ± 12
	8	2	2.8 ± 0.8	54 ± 16	3.2 ± 0.8	31 ± 8	4.0 ± 1.1	23 ± 12	3.9 ± 0.7	18 ± 4
	7	15	3.8 ± 1.2	37 ± 14	4.4 ± 1.3*	23 ± 11	5.6 ± 1.5	15 ± 7	6.7 ± 2.7	11 ± 6
Limb muscle	10	0	1.8 ± 0.8	100 ± 37	2.3 ± 0.8	60 ± 30	2.7 ± 0.8	41 ± 26	4.7 ± 1.8	20 ± 9
	8	2	2.6 ± 0.5	56 ± 14	3.2 ± 0.6	31 ± 10	4.2 ± 1.5	25 ± 11	4.1 ± 0.9	18 ± 6
	7	15	2.5 ± 0.5	53 ± 14	3.0 ± 0.6	31 ± 10	3.4 ± 0.7	24 ± 8	3.8 ± 0.7	17 ± 7
Kidney	10	0	212 ± 65	0.90 ± 0.3	271 ± 53	0.45 ± 0.1	258 ± 48*	0.37 ± 0.1	253 ± 70*	0.35 ± 0.1
	8	2	199 ± 61	0.77 ± 0.2	210 ± 76*	0.48 ± 0.1	243 ± 92*	0.38 ± 0.1	227 ± 73*	0.36 ± 0.1
	7	15	280 ± 98	0.51 ± 0.2	256 ± 62*	0.36 ± 0.1	269 ± 100*	0.32 ± 0.1	281 ± 73*	0.23 ± 0.1
Respiratory rate (breaths/min)	10	0	—		—		—		—	
	8	2	27 ± 7		31 ± 9*		31 ± 7*		30 ± 7*	
	7	15	33 ± 7		33 ± 7*		37 ± 12*		37 ± 13*	
Blood pressure (mm Hg)	10	0	169 ± 33		118 ± 21		97 ± 28		84 ± 23	
	8	2	142 ± 16		95 ± 19		84 ± 20		69 ± 14	
	7	15	128 ± 14		83 ± 26		77 ± 19		58 ± 21	

* Not different from control value ($P > 0.05$). All other values different from control ($P < 0.05$). Q = flow [ml/(min × 100 g)]. R = resistance [mm Hg/ml/(min × 100 g)].

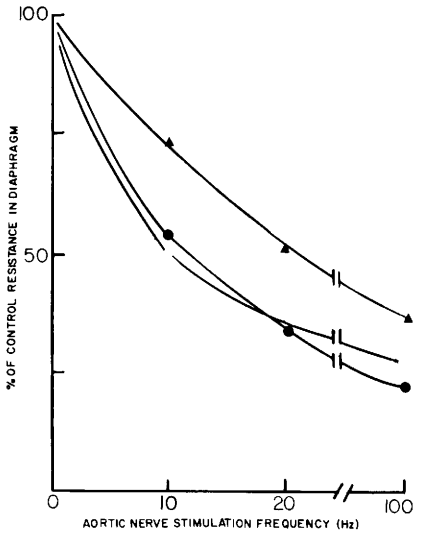

FIG. 2. Resistance changes in the diaphragm to aortic nerve stimulation. Resistance changes expressed relative to the resistance observed with no stimulation. Open circles: quiet breathing with no added inspiratory load. PIP = 2 mm Hg. Closed circles: animals paralyzed with Flaxedil and on respirator. Triangles: breathing against inspiratory load. PIP = 15 mm Hg.

showed the expected increase and regional vascular resistance decreased as expected with increased respiratory effort. Blood flow to the noncontracting diaphragm (PIP = 0) was only 1/10 that seen during normal quiet breathing (PIP = 2 mm Hg) and less than 1/20 of the flow observed with inspiratory loading (PIP = 15 mm Hg). Flow to the intercostal muscles also increased in a similar but less dramatic manner. In contrast to the data presented in Table I flow to limb muscle and kidney did not change in a consistent manner as inspiratory load was increased. Systemic pressure was also higher in this group of animals than in those presented in Table I. We have no explanation for these differences since control conditions, except in the paralyzed animals, were similar.

Stimulation of the AN caused the expected fall in systemic pressure in all animals. With maximum stimulation (100 Hz) blood pressure was decreased to about 50% of control value. This decrease in pressure was not influenced by the level of inspiratory load used in these experiments.

Regional flow changes caused by AN stimulation will depend on both the change in systemic pressure and the change in regional vascular resistance. At all levels of inspiratory load flow increased ($P < 0.05$) in the respiratory and limb muscle vascular beds at the higher levels of AN stimulation although systemic pressure was reduced. Flow to the kidneys was unchanged during AN stimulation. Regional vascular resistance was decreased by AN stimulation in all beds.

In the paralyzed animals the base level of vascular resistance in the diaphragm was 78 ± 25 U [U = mm Hg/ml/(min × 100g)]. Maximum AN stimulation (100 Hz) reduced this 79% to 16 ± 6 units. In the animals breathing quietly the base level of diaphragmatic resistance was 5.1 ± 1 units. Maximum AN stimulation reduced this 72% to 1.4 ± 0.5 units. The base level of diaphragmatic resistance was 2.3 ± 0.3 units in the animals breathing against an inspiratory load (PIP = 15 mm Hg). Maximum AN stimulation further reduced this resistance by 66% to 0.8 ± 0.2 units. Figure 2 illustrates the stimulus-resistance response curves in the diaphragm to AN stimulation at the different levels of respiratory activity. Nearly identical response curves were obtained in the paralyzed animals and in the animals breathing quietly with no added inspiratory load. Adding an inspiratory load shifted this response curve upward. However, the difference between this curve and the one obtained with no added resistance was only significant ($P < 0.05$) at the stimulus frequency of 10 Hz. Response curves at higher levels of inspiratory load were not obtained for the reasons previously mentioned. Flow and resistance changes in the intercostal muscles paralleled those described for the diaphragm.

Resistance in the skeletal muscle of the forelimb and in the kidney were reduced by stimulation of the AN. In the kidney the maximum reduction was 55–60% while in forelimb muscle a 70% reduction occurred. Inspiratory load did not change the base level of resistance or the response to AN stimulation in these organs in any consistent manner.

Discussion. In the present experiments we observed a threefold increase in diaphragmatic flow and a doubling of intercostal flow at the highest level of inspiratory resistance used. Systemic arterial pressure was essentially unchanged in these experiments therefore vasodilation must have occurred in these beds. It is unlikely that this vasodilation results from the activation of reflexes arising from the aorta, carotid sinus, or cardiopulmonary region since both vagi and both AN were cut and both carotid arteries were occluded. It is also unlikely that occlusion of both carotid arteries reduced blood flow in the brain to a degree that would completely alter the neural regulation of the vascular system. In an earlier study on rabbits (4) in which one carotid artery was not occluded but otherwise were similar to those in the present study we observed control regional flows somewhat higher than those in the present study. In an unpublished study on sinoaortic denervated rabbits (N = 13) with one patent carotid artery and intact vagi, flow to the diaphragm and intercostal muscles averaged 40 ± 17 and 4.1 ± 2.4 ml/(min × 100g), respectively. These results suggest that the control levels of regional blood flow in the present experimental preparation are not grossly different from those seen in a similar preparation where one carotid artery is patent and little reduction in brain flow would be expected.

A second possibility that may have influenced the response to inspiratory loading is the high level of sympathetic vascular tone that is expected in these animals due to the reduction in baroreflex activity. In the three animals with intact AN and vagi and one patent carotid artery, inspiratory loading increased flow in the respiratory muscles, although to a somewhat lesser degree, as in the debuffered animals. It seems likely that the increased flow to the respiratory muscles during inspiratory loading primarily results from local metabolic changes rather than changes in the neural regulation of the vascular beds.

Several studies have demonstrated a relationship between blood flow and respiratory muscle activity. Robertson *et al.* (6, 7) have shown in dogs that flow to the diaphragm increased exponentially as the work load was increased by increasing inspiratory resistance. However, during nonresistive increases in work during hyperventilation a linear relationship was observed. Flow to the respiratory muscles increases during nonresistive hypoxic or hypercapnic hyperventilation in rabbits (3–5) and dogs (1, 2, 6, 7). The vasodilation which occurs in the respiratory muscles under the above conditions is probably of metabolic origin rather than the result of reflex effects. We have observed in an earlier study (4) that the vasodilation seen during hypoxia or hypercapnia does not occur in rabbits paralyzed with Flaxedil where metabolic activity in the respiratory muscles is unchanged.

The AN of the rabbit has been shown to primarily arise from aortic baroreceptors (12). Stimulation of this nerve decreases blood pressure due primarily to a reduction of sympathetic vasoconstrictor activity (13). The reduction in the diaphragmatic vascular resistance during AN stimulation was similar in magnitude at the three levels of inspiratory load we used in these experiments (see Fig. 2). In the inactive respiratory muscle we would expect local metabolic regulation of vascular resistance to be minimal and neural effects maximal. At higher levels of respiratory activity the metabolic influence should increase and the neural control should diminish in importance. The effect of this change in the balance between these regulatory factors should shift the response curve to AN stimulation upward from that seen in the inactive state. At the highest level of inspiratory load used, there was some shift in the AN response curve from that observed in the paralyzed animals but the difference between these curves was not great. We would expect this difference to increase at still higher inspiratory loads but were unable to obtain this information because respiratory failure rapidly ensued if the higher loads were maintained for the time necessary for the AN stimulation series.

The relative importance of neural and local metabolic factors in the regulation of blood flow to resting and active limb

skeletal muscle has been the subject of several studies. Experiments in dogs (14–16) and cats (17) have indicated that neural factors play a minor role in regulating vascular resistance of active limb skeletal muscle. However, Vatner *et al.* (16) have shown in conscious, exercising dogs that carotid sinus nerve stimulation during exercise caused further dilation in the metabolically dilated iliac bed. Rowlands and Donald (14) demonstrated that stimulation of sympathetic nerves caused a frequency and time-dependent response in the exercising limb muscle which could be less or greater than the response in the nonexercising muscle. Most studies indicate that the neural factors serve a predominant role in the vascular regulation in inactive limb muscle while local factors predominate during activity. This probably is the case in respiratory muscle also but changes in sympathetic activity appears to be capable of influencing the respiratory muscle vasculature over a wide range of respiratory activity.

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