Renin-Angiotensin System Inhibition in Conscious Dogs during Acute Hypoxemia

EFFECTS ON SYSTEMIC HEMODYNAMICS, REGIONAL BLOOD FLOWS, AND TISSUE METABOLISM

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ABSTRACT The role of the renin-angiotensin system in mediating the circulatory and metabolic responses to hypoxia was studied in three groups of conscious dogs that were infused continuously with normal saline, teprotide (10 μg/kg per min), and saralasin (1 μg/kg per min), respectively. Hypoxia was produced by switching from breathing room air to 5 or 8% oxygen-nitrogen mixture. Plasma renin activity increased from 2.3±0.4 to 4.9±0.8 ng/ml per h during 8% oxygen breathing, and from 2.8±0.4 to 8.4±1.8 ng/ml per h during 5% oxygen breathing. As expected, cardiac output, heart rate, mean aortic blood pressure, and left ventricular *dP/dt* and *dP/dt/P* increased during both 5 and 8% oxygen breathing in the saline-treated dogs; greater increases occurred during the more severe hypoxia. Teprotide and saralasin infusion diminished the hemodynamic responses to 5% oxygen breathing, but did not affect the responses to 8% oxygen breathing significantly. In addition, the increased blood flows to the myocardium, kidneys, adrenals, brain, intercostal muscle, and diaphragm that usually occur during 5% oxygen breathing were reduced by both agents. These agents also reduced the increases in plasma norepinephrine concentration during 5% oxygen breathing, but had no effects on tissue aerobic or anaerobic metabolism.

In dogs pretreated with propranolol and phentolamine, administration of teprotide (0.5 mg/kg) during 5% oxygen breathing reduced mean aortic blood pressure and total peripheral vascular resistance, and increased cardiac output and heart rate, but did not affect left ventricular *dP/dt*, *dP/dt/P*, and end-diastolic pressure. Simultaneously, renal and myocardial blood flows increased and myocardial oxygen extraction decreased, while myocardial oxygen consumption did not change significantly.

These results suggest that the renin-angiotensin system plays an important role in the hemodynamic responses to severe hypoxia. It appears that angiotensin not only exerts a direct vasoconstrictor action, especially upon the coronary and renal circulations, but also potentiates the cardiovascular effects of sympathetic stimulation that occur during severe hypoxia.

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INTRODUCTION

Increasing evidence has accumulated that hypoxia stimulates the renin-angiotensin system. Low oxygen tension increases the granularity of human juxtaglomerular cells cultured in vitro (1). Alveolar hypoxia in vivo also increases the number of granules of juxtaglomerular cells (2, 3), plasma renin activity (4, 5), plasma angiotensin II levels (6), and the angiotensin converting enzyme activity both in the lungs and in the serum (7). The mechanisms of renin release during hypoxia are not clearly elucidated. The increase in the granularity of juxtaglomerular cells (1–3) would suggest a direct action of hypoxia on renin release, but acute local renal perfusion with hypoxic blood did not increase the renin activity in renal venous blood (8). On the other hand, renin may be released by sympathetic discharge (9) produced by hypoxia. Furthermore, renal blood flow may decrease during severe hypoxia and this can also cause renin release (9). The magnitude

of the renin-angiotensin response to hypoxia may also be related to the degree of hypoxia and the underlying state of activation of the renin-angiotensin system (6).

In this paper, we studied the physiologic importance of the renin-angiotensin system in mediating the cardiovascular responses to both moderate and severe hypoxia by pretreating animals with teprotide (SQ 20,881), an angiotensin converting enzyme inhibitor (10), and saralasin (1-sarcosine-8-alanine-angiotensin II), an angiotensin-receptor blocking agent (11). Regional blood flows and anaerobic tissue metabolism were also studied. We have recently found that administration of teprotide increases blood flow to the myocardium, kidneys, and brain in conscious dogs during salt depletion (12, 13). This suggests that these organs are particularly susceptible to the vasoconstrictor action of angiotensin II.

Because angiotensin could have a significant action on the sympathetic nervous system (14), it may exert its hemodynamic effects indirectly by increasing catecholamine release. To study the possible contribution of the sympathetic nervous system during hypoxia, we measured plasma norepinephrine levels in dogs with and without the renin-angiotensin system inhibition. In addition, we administered teprotide into dogs during hypoxia after they had been pretreated with α- and β-adrenergic receptor blocking agents to study the direct effects of angiotensin on the cardiovascular system during hypoxia.

METHODS

Adult male beagle dogs, weighing between 6.8 and 16.8 kg, were sedated with morphine sulfate (1 mg/kg). The trachea was cannulated with a T tube, by local lidocaine (Xylocaine) anesthesia. The aorta and the coronary sinus were cannulated with French 8 Cournand catheters, and the pulmonary artery was cannulated with a French 7 Swan-Ganz catheter (Edwards Laboratories, Inc., Santa Ana, Calif.). The aortic catheter was introduced via a femoral artery. Both coronary sinus and Swan-Ganz catheters were inserted under fluoroscopic guidance via external jugular veins. All catheters were connected to Statham P23Db pressure transducers (Statham Instruments, Inc., Oxnard, Calif.), whose signals were fed into a multichannel Brush 480 recorder (Gould, Inc., Instrument Systems Division, Cleveland, Ohio) to measure blood pressures and heart rate.

The left ventricle was cannulated via the left carotid artery with a Millar transducer-tip catheter (Millar Instruments, Inc., Houston, Tex.) for measuring left ventricular end-diastolic and systolic pressures, and the first derivative of left ventricular pressure (dP/dt). The ratio of dP/dt to a developed left ventricular pressure of 50 mm Hg was calculated for 10 consecutive cardiac cycles at each measurement period, with a PDP-11/10 minicomputer (Digital Equipment Corp., Maynard, Mass.). This pressure occurred during isovolumic systole; this ratio is referred to as $dP/dt/P$ (15).

Cardiac output was determined by an indocyanine green (Cardio-Green) dye dilution technique, with a Gilford model 140 cardiac output system (Gilford Instrument Laboratories Inc., Oberlin, Ohio). Regional blood flows were determined by a modification (16) of the radioactive microsphere method of Rudolph and Heymann (17). 450,000 microspheres, 15±3 μm in diameter, and labeled with cerium-141, tin-113, strontium-85, or scandium-46 at a sp act of 10 mCi/g, were injected into the left ventricle via the Millar catheter, which was immediately flushed with 10 ml of normal saline over a 30-s period. Arterial reference blood was withdrawn with a Harvard pump (Harvard Apparatus Co., Inc., Millis, Mass.) at a rate of 7.75 ml/min, beginning 10 s before the injection of microspheres and continuing for 80 s thereafter. The content of radioactivity in each organ was measured in a Packard gamma spectrometer with a model 9012 multichannel analyzer (Packard Instrument Co., Inc., Downers Grove, Ill.) at the appropriate gamma photon energy for each of the radionuclides. Absolute blood flow to each organ was calculated by the reference sample method on a PDP-11/10 minicomputer (16). Mean aortic blood pressure was divided by cardiac output or organ blood flow to yield the total peripheral or regional organ vascular resistance.

Arterial and coronary sinus venous blood samples were obtained to measure pH, P_{O_2} and P_{CO_2} on a Radiometer PHM71 acid base analyzer (Radiometer Co., Copenhagen, Denmark), and to measure oxygen content by gas chromatography (18). Blood oxygen capacity was measured by a cyanmethemoglobin method (19). Plasma renin activity (20) and norepinephrine concentration (21) were determined by radioimmunoassay and radioenzymatic methods, respectively. Arterial and coronary blood samples were taken immediately after microsphere injections for measuring lactate (22), pyruvate (23), β-hydroxybutyrate (24), and acetoacetate (25). Myocardial oxygen consumption, left ventricular work, total body oxygen consumption, and myocardial utilization rates of lactate and pyruvate were calculated by conventional formulas.

21 animals were divided equally into three groups. They were infused continuously with normal saline, teprotide, or saralasin at a rate of 0.229 ml/min, delivered by a Harvard infusion pump (Harvard Apparatus Co., Inc.). Teprotide and saralasin were dissolved in normal saline in concentrations which permitted delivery of 10 μg/kg per min and 1 μg/kg per min, respectively. The protocol involved an initial 20-min control period, a 20-min period of hypoxia, a 40-min recovery period, a second 20-min control period, a second 20-min period of hypoxia, and a final recovery period. The normal saline or drug infusion continued throughout the experiment, and each dog received only one kind of infusion. Two different degrees of hypoxia were produced in each dog during the two hypoxic periods by switching from breathing room air to 8 or 5% oxygen-nitrogen mixture. The sequence of these two degrees of hypoxia varied in random order from dog to dog. Systemic hemodynamic measurements were obtained at 5-min intervals during both control and hypoxic periods. Averages were obtained from triplicate measurements of each hemodynamic variable during the control period. These values from each dog were then averaged and are reported as prehypoxic control values in Results. Circulating tissue metabolites, plasma renin activity, plasma norepinephrine, and organ blood flows were determined immediately before hypoxia and after 20 min of hypoxia, 1 μg [1-Asp, 5-Ile]angiotensin I (Schwartz/Mann Div., Becton, Dickinson and Company, Orangeburg, N. Y.) and 1 μg [1-Asp, 5-Ile]angiotensin II (Sigma Chemical Co., St. Louis, Mo.) were administered intravenously, before the start of the normal saline or drug infusion and again during the recovery periods after hypoxia, to ascertain whether pharmacologic blockade had been produced by teprotide and saralasin.

Another 23 dogs, also divided into three groups, were pretreated intravenously with propranolol (0.3 mg/kg) and

phentolamine (5 mg/kg). Degrees of α- and β-adrenergic receptor blockade were determined, before and after drug treatments, by measuring aortic blood pressure and heart rate responses to norepinephrine and isoproterenol, respectively. Two groups of the dogs were made hypoxic with 5% oxygen breathing for 20 min and after 10 min of hypoxia, either teprotide (0.5 mg/kg) or normal saline (2 ml) was injected. The same does of teprotide was administered to the third group that breathed room air. Systemic hemodynamic measurements were also obtained every 5 min. Regional blood flows and metabolites were measured during the control period, and at 10 min and 20 min of hypoxia. Angiotensin I (1 μg) was administered intravenously at the beginning and end of the experiment to determine angiotensin converting enzyme inhibition.

The data for each degree of hypoxia were treated statistically with two-way analysis of variance for independent groups with trend analysis (26), and the significance of the differences between control and experimental values was determined by Dunnett's test (27). The difference was considered significant if $P < 0.05$. Student's t test for paired comparisons was used to determine the statistical significance of a difference between two means in the same group of animals. Values are mean±SE.

RESULTS

Effects of alveolar hypoxia on arterial blood gases and plasma renin activity. Acute alveolar hypoxia decreased arterial blood P_{O_2} and P_{CO_2}, and increased arterial blood pH and plasma renin activity. The magnitude of changes was similar in all three groups of dogs treated with normal saline (11.1±1.0 kg), teprotide (11.9±1.2 kg), and saralasin (12.2±1.0 kg), and the results were pooled for statistical analysis (Table I). As expected, the more severe hypoxia produced greater changes in these parameters.

Effects of teprotide and saralasin on pressor responses to angiotensin I and II. Angiotensin I (1 μg) was injected intravenously before administration of teprotide, and again during teprotide infusion when the hemodynamic measurements had returned to baseline values, after 8 and 5% oxygen breathing. Mean aortic blood pressure in response to angiotensin I increased 20±4 mm Hg before teprotide administration, but increased only 5±1 and 8±2 mm Hg after 8 and 5% oxygen breathing, respectively. Similar administration of angiotensin II increased mean aortic blood pressure 36±3 mm Hg before saralasin administration, but, during saralasin infusion, only 3±1 and 1±1 mm Hg after 8 and 5% oxygen breathing, respectively. These decreases in pressor responses to angiotensin I and II after administration of teprotide and saralasin, respectively, were significant at $P < 0.001$, as determined by Student's t test for paired comparisons.

TABLE I
Effects of 8% and 5% Oxygen Breathing on Arterial Blood Gases and Plasma Renin Activity

	Arterial blood			Plasma renin activity
	P_{O_2}	P_{CO_2}	pH	
	mm Hg	*mm Hg*		*ng/ml/h*
8% Oxygen breathing				
Control	86±2	43±1	7.38±0.01	2.3±0.4
Hypoxia	31±1*	28±2*	7.52±0.01*	4.9±0.8*
5% Oxygen breathing				
Control	84±2	42±1	7.39±0.01	2.8±0.4
Hypoxia	23±1*	21±1*	7.58±0.01*	8.4±1.8*

Values are mean±SE; $n = 21$ in each group.
* Indicates values that are significantly different from the prehypoxic control at $P < 0.05$.

Angiotensin I and II were also administered to the saline-treated animals. At the beginning of the experiment, angiotensin I and II increased mean aortic blood pressure 18±3 and 31±2 mm Hg, respectively, and at the end of the experiment 17±1 and 30±2 mm Hg, respectively. There were no significant differences between the pressor responses to angiotensin at the beginning and end of the experiment.

Effects of teprotide and saralasin on changes in systemic hemodynamics and regional blood flow during hypoxia. Alveolar hypoxia increased cardiac output, heart rate, mean aortic blood pressure, left ventricular dP/dt and $dP/dt/P$, and decreased total peripheral vascular resistance. In the saline group, severe hypoxia with 5% oxygen breathing produced greater hemodynamic changes than moderate hypoxia with 8% oxygen breathing (Fig. 1). The control values before hypoxia were similar before 8 and 5% oxygen breathing in this group. Similar values were found in the prehypoxic periods in the teprotide and saralasin groups. Fig. 2 shows the percent change (from the control value) during hypoxia, in each hemodynamic variable for all three experimental groups. The magnitude of the responses to a given degree of hypoxia was similar in each experimental group, regardless of the sequence of administering the 5 and 8% oxygen-nitrogen mixtures. Neither teprotide nor saralasin significantly altered the hemodynamic responses to 8% oxygen breathing, but both agents reduced the increases in cardiac output, heart rate, mean aortic blood pressure, left ventricular dP/dt, and $dP/dt/P$ that occurred during 5% oxygen breathing. Changes in total peripheral vascular resistance, however, did not differ among the groups. In addition, left ventricular end-diastolic pressure decreased in the saline group from 9.0±1.2 to 6.6±1.1, and from 9.1±1.3 to 4.7±0.8 mm Hg during 8 and 5% oxygen breathing, respectively. Similar decreases during 8 and 5% oxygen breathing were found in the teprotide (−3.0±1.0 and −2.2±0.9 mm Hg, respectively) and saralasin (−2.7±0.8 and −2.2±1.0 mm Hg, respectively) groups.

Table II shows the percent changes in regional blood

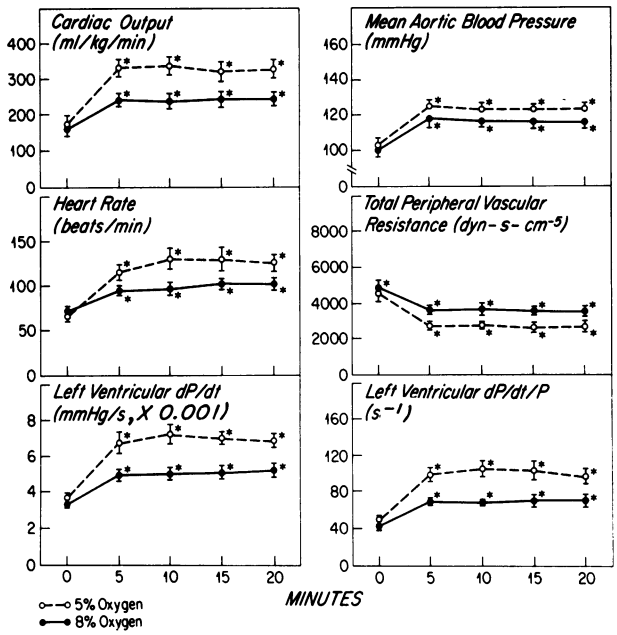

FIGURE 1 Changes in cardiac output, heart rate, mean aortic blood pressure, total peripheral vascular resistance, left ventricular *dP/dt*, and *dP/dt/P* in seven saline-treated conscious dogs during 8% (closed circles) and 5% (open circles) oxygen breathing. Bars show SE. Asterisks indicate values that are statistically different from the prehypoxic values at $P < 0.05$, as determined by Dunnett's test (27).

flows during hypoxia in the saline-, teprotide-, and saralasin-treated animals; the prehypoxic values did not differ among the groups. In the saline group, blood flow increased to the right and left ventricles, adrenal glands, diaphragm, and intercostal muscle during 8% oxygen breathing. Blood flow also increased to the brain, kidneys, liver, and femoral muscle during 5% oxygen breathing. Blood flow to lungs, stomach, small and large intestines, skin, and bone, however, did not change significantly during either gas breathing. Table II also shows that blood flow responses to 8% oxygen breathing in the teprotide and saralasin groups were like those in the saline group, but the increases in blood flows to the ventricles, kidneys, adrenals, brain, intercostal muscle, and diaphragm that occur during 5% oxygen breathing were reduced by teprotide and saralasin.

Effects of teprotide and saralasin on changes in plasma norepinephrine concentration during hypoxia. Plasma norepinephrine concentration did not change significantly during 8% oxygen breathing in the saline-, teprotide-, and saralasin-treated dogs (Table III). In contrast, it increased significantly during 5% oxygen breathing. Its increases, however, were significantly smaller in the teprotide and saralasin groups than in the saline group.

Effects of teprotide and saralasin on changes in cardiac metabolism and energetics during hypoxia. Table IV shows the changes in myocardial oxygen consumption, left ventricular work, myocardial oxygen extraction, and myocardial utilization rates of lactate and pyruvate that occurred during 8 and 5% oxygen breathing in the saline-, teprotide-, and saralasin-treated dogs. The prehypoxic control values of these variables in the saline-treated dogs were 500±98 μmol/100 g per min, 6.8±0.7 kg·m/100 g per min, 78±4%, 87±20 μmol/100 g per min, and 17±4 μmol/100 g per min, respectively. Similar control values were found in the teprotide- and saralasin-treated dogs.

Alveolar hypoxia increased myocardial oxygen consumption, left ventricular work, and myocardial utilization of lactate and pyruvate in the saline-treated animals. These changes were more marked during 5% oxygen breathing than 8% oxygen breathing. Myocardial oxygen extraction, however, did not change at either level of hypoxia.

Infusion of teprotide and saralasin did not modify the cardiac metabolic responses to 8% oxygen breathing, but did reduce the increases in myocardial oxygen consumption, left ventricular work, and myocardial utilization of lactate and pyruvate that occurred during 5% oxygen breathing.

Effects of teprotide and saralasin on changes in total body oxygen consumption and anaerobic tissue

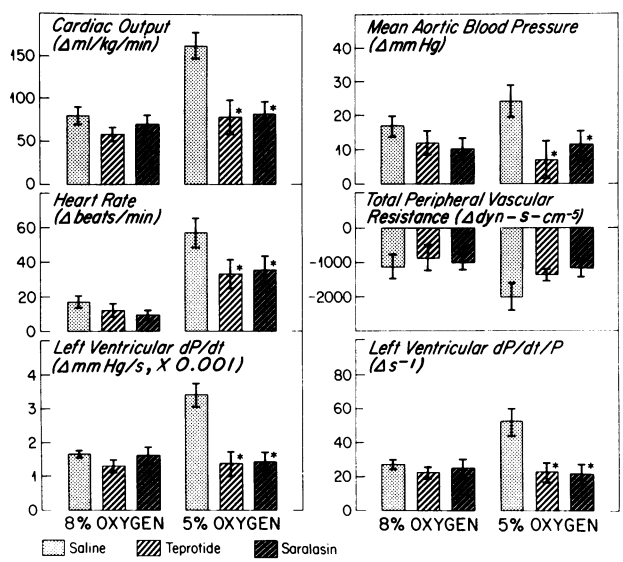

FIGURE 2 Changes in cardiac output, heart rate, mean aortic blood pressure, total peripheral vascular resistance, left ventricular *dP/dt*, and *dP/dt/P* during 8% and 5% oxygen breathing in the saline-, teprotide-, and saralasin-treated dogs ($n = 7$ in each group). The changes were based on the average values of repetitive measurements made at 5-min intervals during the 20-min hypoxic period. Bars show SE. Asterisks indicate values that are statistically different from those in the saline-treated dogs at $P < 0.05$, as determined by Dunnett's test (27).

TABLE II

Percent Changes in Organ Blood Flows during Hypoxia in Saline-, Teprotide-, and Saralasin-Treated Animals

Organ	8% Oxygen breathing			5% Oxygen breathing		
	Saline	Teprotide	Saralasin	Saline	Teprotide	Saralasin
Right ventricle	133±27*	97±26*	71±15*	636±114*	148±25*‡	167±32*‡
Left ventricle	96±20*	98±21*	81±16*	690±170*	194±30*‡	200±34*‡
Kidneys	24±18	7±7	−6±5	77±29*	−10±6‡	2±7‡
Adrenals	100±24*	51±10*	42±16*	276±70*	51±14*‡	63±10*‡
Brain	52±28	26±14	51±20	341±88*	94±25*‡	138±31*‡
Lungs (bronchial)	−3±24	16±18	5±14	137±76	75±39	55±26
Liver (hepatic)	26±19	20±24	10±19	79±18*	79±13*	113±25*
Stomach	23±13	17±24	−28±15	61±30	−15±14	54±27
Small intestine	21±8	−15±20	−17±9	35±33	−11±8	0±7
Large intestine	−9±13	−11±8	−11±9	21±29	15±8	10±16
Skin	25±17	5±21	−10±12	−14±30	−14±11	18±16
Femur	16±16	−6±15	−11±10	22±37	−21±16	−10±5
Muscle						
Femoral	48±19	44±23	21±24	184±33*	87±31*	70±28*
Intercostal	90±31*	88±28*	81±22*	373±39*	172±37*‡	171±28*‡
Diaphragm	110±28*	98±35*	69±19*	947±222*	338±84*‡	387±77*‡

Values are percent changes from the control in mean±SE; $n = 7$ in each group.

* Indicates changes that are significantly different from the prehypoxic control at $P < 0.05$.

‡ Indicates that the value is different from that in the saline-treated dogs at $P < 0.05$, as determined by Dunnett's test (27).

metabolism during hypoxia. Table V shows the hypoxic changes in total body oxygen consumption, arterial lactate concentration, and lactate:pyruvate and β-hydroxybutyrate:acetoacetate ratios in the saline-, teprotide-, and saralasin-treated dogs. The prehypoxic control values of these variables were 8.3±0.5 ml/kg per min, 2.0±0.2 mM, 6.7±0.4, and 1.1±0.1, respectively, in the saline group; the other two groups had similar control values. Total body oxygen consumption did not change during either 8 or 5% oxygen breathing in any of these three groups. Arterial lactate concentration, lactate:pyruvate ratio, and β-hydroxybutyrate: acetoacetate ratio, however, increased, and were larger during 5% oxygen breathing than 8% oxygen breathing. These increases also did not differ among the three groups.

Effects of teprotide on hemodynamic responses to 5% oxygen breathing in propranolol- and phentolamine-pretreated dogs. Propranolol and phentolamine pretreatment reduced heart rate and pressor responses to isoproterenol and norepinephrine, respectively. The dose of isoproterenol required to accelerate heart rate 25 beats/min increased from 1.5±0.3 μg before to 21±2 μg after pretreatment ($P < 0.001$), and the dose of norepinephrine required to raise arterial blood pressure 25 mm Hg increased from 4.6±0.3 to 57±6 μg

TABLE III

Effects of Hypoxia on Plasma Concentration of Norepinephrine

Group	8% Oxygen breathing			5% Oxygen breathing		
	Control	Hypoxia	Change	Control	Hypoxia	Change
	ng/ml			*ng/ml*		
Saline	0.17±0.03	0.42±0.16	0.25±0.13	0.23±0.05	0.90±0.22*	0.67±0.14
Teprotide	0.31±0.06	0.44±0.16	0.13±0.06	0.31±0.06	0.63±0.13*	0.32±0.08‡
Saralasin	0.22±0.04	0.34±0.09	0.12±0.08	0.27±0.06	0.57±0.11*	0.29±0.09‡

Values are mean±SE; $n = 7$ in each group.

* Indicates values that are significantly different from the prehypoxic values at $P < 0.05$.

‡ Indicates that the hypoxic change is significantly different from that in the saline-treated dogs at $P < 0.05$, as determined by Dunnett's test (27).

TABLE IV
Changes in Cardiac Metabolism and Energetics during Hypoxia in Saline-, Teprotide-, and Saralasin-Treated Animals

Group	Myocardial oxygen consumption	Left ventricular work	Myocardial oxygen extraction	Myocardial utilization	
				Lactate	Pyruvate
	μmol/100 g/min	*kg·m/100 g/min*	%	*μmol/100 g/min*	
8% Oxygen breathing					
Saline	130±66	4.4±0.5	4±4	38±10	7±2
Teprotide	156±44	2.6±0.3	7±4	35±8	8±3
Saralasin	112±40	3.9±1.1	4±3	40±11	10±3
5% Oxygen breathing					
Saline	420±73	7.1±0.8	2±4	79±14	24±4
Teprotide	89±44*	3.0±1.0*	−4±6	36±6*	6±2*
Saralasin	134±51*	4.0±0.7*	2±2	43±8*	12±2*

Values are changes from control values in mean±SE; $n = 7$ in each group. All the changes produced by hypoxia, except those in myocardial oxygen extraction, are statistically significant at $P < 0.05$.
* Indicates that the hypoxic change is significantly different from that in the saline-treated dogs at $P < 0.05$, as determined by Dunnett's test (27).

($P < 0.001$). 5% oxygen breathing reduced arterial blood P_{O_2} from 90±4 to 21±1 and 22±2 mm Hg, at 10 and 20 min of hypoxia, respectively. Cardiac output, heart rate, left ventricular *dP/dt*, and *dP/dt/P* increased, and total peripheral vascular resistance decreased during hypoxia, but mean aortic blood pressure did not change significantly (Fig. 3). Administration of teprotide at 10 min of hypoxia in nine dogs (10.9±0.7 kg) reduced mean aortic blood pressure and total peripheral vascular resistance, and further increased cardiac output and heart rate. Left ventricular *dP/dt* and *dP/dt/P*, however, were not affected. Left ventricular end-diastolic pressure decreased from 6.1±1.2 to 3.9±1.1 and 3.3±1.2 mm Hg at 10 and 20 min of hypoxia. The pressor response to angiotensin I (1 μg) was reduced from 21±1 to 4±1 mm Hg ($P < 0.001$) after teprotide administration. Fig. 3 also shows that similar administration of normal saline during hypoxia in nine dogs (12.3±1.6 kg) did not produce any significant hemodynamic changes. In the latter group, plasma renin activity increased significantly from 1.1±0.3 to 5.3±0.7 ng/ml per h after 20 min of hypoxia.

TABLE V
Changes in Aerobic and Anaerobic Metabolism during Hypoxia in Saline-, Teprotide-, and Saralasin-Treated Animals

Group	Total body oxygen consumption	Arterial lactate concentration	Lactate: pyruvate	β-Hydroxybutyrate: acetoacetate
	ml/kg/min	*mM*		
8% Oxygen breathing				
Saline	−0.6±0.3	1.0±0.3	1.6±0.4	0.3±0.1
Teprotide	0.6±0.3	1.0±0.2	2.1±0.8	0.5±0.2
Saralasin	−0.2±0.3	1.7±0.2	1.8±0.4	0.3±0.1
5% Oxygen breathing				
Saline	0.6±0.4	4.2±0.8	5.3±1.5	1.6±0.5
Teprotide	0.3±0.3	4.2±0.4	6.0±0.8	1.5±0.5
Saralasin	−0.5±0.8	4.3±0.4	4.6±0.7	1.5±0.4

Values are changes from control values in mean±SE; $n = 7$ in each group. Changes in total body oxygen consumption during hypoxia are not statistically significant, whereas changes in arterial lactate concentration, lactate:pyruvate ratio and β-hydroxybutyrate:acetoacetate ratio are all significant at $P < 0.05$. None of these changes differ among the groups.

Teprotide was administered to five propranolol- and phentolamine-pretreated dogs (13.1±1.2 kg) that breathed room air. It did not change mean aortic blood pressure (from 97±7 to 96±7 mm Hg), cardiac output (from 2.87±0.37 to 2.98±0.39 liters/min), left ventricular *dP/dt* (from 2,874±106 to 2,958±140 mm Hg/s), and *dP/dt/P* (from 41±2 to 41±3 s^{-1}).

Table VI shows regional blood flows before and after teprotide administration during 5% oxygen breathing. Teprotide administration increased blood flow to the ventricles and kidneys, while the blood flow to other organs did not change significantly. Concomitantly, organ vascular resistance decreased significantly in the heart, kidneys, stomach, and small and large intestines by 39±9, 36±3, 24±6, 48±9, and 41±6%, respectively. In contrast, neither blood flow nor organ vascular resistance changed significantly after saline administration during hypoxia. Blood flows in the right ventricle, left ventricle, and kidneys were 210±22, 310±30, and 531±33 ml/100 g per min, respectively, before saline administration, and were 218±25, 305±27, and 505±70

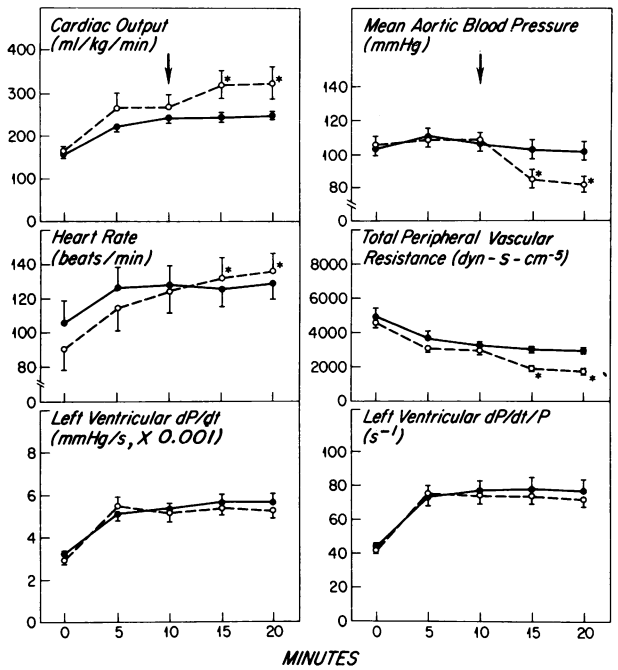

FIGURE 3 Changes in cardiac output, heart rate, mean aortic blood pressure, total peripheral vascular resistance, left ventricular *dP/dt*, and *dP/dt/P* during 5% oxygen breathing in two groups of animals that had been pretreated with propranolol and phentolamine. At 10 min of hypoxia, indicated by arrows, teprotide (0.5 mg/kg) was administered intravenously to one group of nine dogs (open circles) and normal saline (2 ml) to the other group (closed circles, $n = 9$). Bars show SE. Asterisks indicate values that are statistically different from the averages of values obtained at 5 and 10 min of hypoxia at $P < 0.05$, as determined by Dunnett's test (27).

ml/100 g per min, respectively, after saline administration.

Effects of teprotide on changes in cardiac metabolism and energetics, and tissue anaerobic metabolism during 5% oxygen breathing in propranolol- and phentolamine-pretreated dogs. Myocardial oxygen consumption and left ventricular work increased during 5% oxygen breathing in propranolol- and phentolamine-pretreated dogs (Table VII). These measurements were not affected by either teprotide or normal saline administered at 10 min of hypoxia, but myocardial oxygen extraction decreased after teprotide administration. Total body oxygen consumption did not change in these animals during hypoxia, nor was it affected by teprotide administration. Furthermore, arterial lactate concentration increased from 2.2±0.3 to 6.9±1.0 mM and lactate:pyruvate ratio from 7.2±0.8 to 14.4±1.2 during hypoxia in the group that received teprotide. Comparable increases in arterial lactate concentration (from 2.6±0.4 to 7.9±0.8 mM) and lactate:pyruvate ratio (from 8.9±1.0 to 15.6±1.4) occurred in the group that received normal saline.

DISCUSSION

Our present study has demonstrated that plasma renin activity increases during acute alveolar hypoxia, and that the renin-angiotensin system inhibition with either teprotide or saralasin significantly reduces the increases in cardiac output, myocardial contractility, mean aortic blood pressure, and heart rate that occur during 5% oxygen breathing. The changes that occur during 8% oxygen breathing are not altered significantly. These results suggest that the renin-angiotensin system plays an important role in mediating the hemodynamic responses to severe hypoxia.

In addition to direct vasoconstrictor and cardiac inotropic effects, angiotensin has been shown to stimulate central vasomotor neurons, to increase release of catecholamines from the adrenal medulla, and to potentiate the cardiovascular responses to sympathetic nerve stimulation and administration of sympathomimetic amines (14). This potentiation of adrenergic stimuli by angiotensin probably is attributable to enhanced synthesis and release, as well as to inhibition of neuronal re-uptake, of norepinephrine at sympathetic nerve terminals (14, 28, 29). The present study shows that the increase in plasma norepinephrine concentra-

TABLE VI
Effects of Teprotide Administration during 5% Oxygen Breathing on Organ Blood Flows in Five Propranolol- and Phentolamine-Pretreated Dogs

Organ	5% Oxygen breathing	
	Before teprotide	After teprotide (0.5 mg/kg)
	ml/100 g/min	
Right ventricle	234±48	329±39*
Left ventricle	317±50	409±42*
Kidneys	516±58	654±73*
Adrenals	1,046±102	1,034±113
Brain	137±17	121±12
Lungs (bronchial)	164±25	113±12
Liver (hepatic)	43±18	52±13
Stomach	67±12	75±16
Small intestine	46±5	80±12
Large intestine	38±3	56±10
Skin	1.4±0.4	1.7±0.4
Femur	3±1	3±1
Muscle		
Femoral	3±1	5±2
Intercostal	16±4	20±6
Diaphragm	40±6	46±6

Values are mean±SE.
* Indicates values that are significantly different from those obtained before teprotide administration at $P < 0.05$, as determined by Student's t test for paired comparisons.

TABLE VII

Effects of Hypoxia and Teprotide on Cardiac Metabolism and Total Body Oxygen Consumption in Dogs Pretreated with Propranolol and Phentolamine

Group	Myocardial oxygen consumption	Left ventricular work	Myocardial oxygen extraction	Total body oxygen consumption
	μmol/100 g/min	*kg·m/100 g/min*	*%*	*ml/kg/min*
Teprotide (n = 5)				
Control	496±45	6.2±0.5	84±9	7.5±0.4
5% O_2	616±49*	11.0±1.4*	90±6	7.3±0.4
5% O_2 + teprotide	630±94*	11.2±1.3*	76±4‡	8.2±0.5
Normal saline (n = 9)				
Control	509±40	6.1±0.4	84±2	8.1±0.7
5% O_2	580±49*	10.1±0.9*	85±3	7.7±0.6
5% O_2 + saline	585±71*	10.2±1.1*	86±3	7.0±0.8

Values are mean±SE.

* Indicates values that are significantly different from the prehypoxic control values at $P < 0.05$.

‡ Indicates the value that is statistically different from the hypoxic value obtained before teprotide administration at $P < 0.05$, as determined by Dunnett's test (27).

tion during severe hypoxia was reduced by teprotide and saralasin. Similarly, saralasin has been shown to reduce the increase in plasma norepinephrine that occurs during strenuous exercise (30). Thus, it appears that endogenous angiotensin may potentiate the effects of sympathetic stimulation that occur physiologically during severe hypoxia and exercise. These results further suggest that the diminished cardiovascular responses to severe hypoxia demonstrated in the present study with teprotide and saralasin infusion could be explained, at least in part, by abolition of the potentiated sympathetic effects.

The direct vasoconstrictor action of angiotensin during hypoxia was documented by a depressor response after teprotide administration in the propranolol- and phentolamine-pretreated dogs. Teprotide also decreased total peripheral vascular resistance, and increased cardiac output and heart rate. Myocardial contractility and left ventricular end-diastolic pressure, however, did not change. The increase in cardiac output after teprotide administration probably was caused, at least in part, by the decrease in aortic blood pressure (31). Teprotide also increased renal and myocardial blood flows. This increase in myocardial blood flow was associated with a decrease in myocardial oxygen extraction; neither myocardial oxygen consumption nor left ventricular work changed significantly. In addition, vascular resistance decreased significantly in the heart, kidneys, and the gastrointestinal tract. These results suggest that angiotensin II exerts an active vasoconstrictor action, especially on the heart, kidneys, and the gastrointestinal tract, during hypoxia, but has no significant effects on myocardial contractility or energetics independent of its effects on the sympathetic nervous system.

The increases in absolute organ blood flows during severe hypoxia were smaller in the teprotide- and saralasin-treated dogs than in the saline-treated controls (Table II). These differences could be attributed, in part, to the differences in cardiac output during hypoxia. When blood flows are recalculated as percents of cardiac output, the differences in blood flow responses among the groups disappear, except for the increase in myocardial blood flow which remains larger in the saline group than in the other two experimental groups. The larger increases in myocardial blood flow and utilization of lactate and pyruvate in the saline group during severe hypoxia probably were related to the higher left ventricular work and myocardial oxygen consumption in that group.

Our study shows that arterial lactate:pyruvate and β-hydroxybutyrate:acetoacetate ratios increased during both 8 and 5% oxygen breathing. The increases were larger during 5% oxygen breathing, indicating a greater degree of anaerobic metabolism during more severe hypoxia. Neither of these ratios, however, was affected by teprotide or saralasin infusion. Total body oxygen consumption also did not change significantly during hypoxia. These results suggest that the diminished cardiac output and blood flow responses to hypoxia in the saralasin- and teprotide-treated dogs did not adversely affect the tissue oxidative metabolism, nor did they exaggerate anaerobic metabolism during 20 min of hypoxia. The continuing myocardial use of lactate during hypoxia in the teprotide and saralasin groups also indicates that no significant myocardial hypoxia oc-

curred in these animals. These changes in hemodynamic and metabolic responses to hypoxia also are produced by pretreatment with propranolol (32). The lower concentrations of norepinephrine in the teprotide- and saralasin-treated animals during hypoxia probably produced a smaller calorigenic effect than that in saline-treated dogs, and thus offset the potential effects of the diminished cardiac output or blood flow response on anaerobic metabolism.

Teprotide is known to inhibit kininase II and augment the effects of bradykinin (10). Results of the present study, however, do not allow us to define the role of this bradykinin-potentiating effect of teprotide on the hemodynamic and metabolic responses to hypoxia. Nevertheless, because both teprotide and saralasin produced similar changes, their effects probably are caused primarily by blockade of the renin-angiotensin system.

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