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Decrease in Renal Perfusion, Glomerular Filtration and Sodium Excretion by Hypoxia in the Dog (40372)

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The present investigation was designed to assess the effect of hypoxia on renal hemodynamics and sodium excretion. Previous studies in dogs have shown conflicting results (1–4). In most studies lowered concentrations of oxygen were administered without controlling ventilation. Presumably, the hyperventilation induced by hypoxia altered blood pH and cardiac output (5); two factors which are known to alter renal function (6–9). Since the pH and pCO_2 were neither measured nor controlled in these studies altered states of acid-base balance might explain the disparate results. In the present study ventilatory rate was maintained constant so that no changes in pCO_2 or pH occurred.

Furthermore, in the earlier studies in which hypoxia reduced GFR and RPF (2, 4) it was not determined whether the altered renal perfusion was due to ischemic injury with cell swelling or to a functional and reversible increase in renal vascular resistance. To determine whether hypoxia exerts a direct toxic effect on the renal vasculature the vasodilator acetylcholine was infused into one renal artery during hypoxia and the GFR, RPF, and sodium excretion of this kidney was compared with the non infused kidney.

Materials and methods. Studies were performed on 12–18 kg mongrel dogs. Food and water were withheld for 2–6 hr before study. All dogs were anesthetized with pentobarbital, intubated and placed on a Harvard 614 respirator. A polyethylene catheter was inserted into a foreleg vein for infusion of inulin or ^{125}I iodothalmate (Glo-fil, Abbott Laboratories), and PAH. Plasma inulin was maintained at approximately 20 mg/100 ml and PAH at 2 mg/100 ml. Blood pressure was monitored and blood obtained for clearance determinations from a femoral artery catheter. Both ureters were catheterized near the renal pelvis through bilateral flank incisions. A large polyvinyl catheter for microsphere injection was advanced through the right axillary or right carotid artery to the aortic root. In the eight animals receiving acetylcholine a hooked 23 gauge needle was placed in the right renal artery and kept open with a saline infusion at a rate of 0.11 ml/min. In four additional animals not receiving acetylcholine a hooked 23 gauge needle was placed in the right renal vein and passed to the hilus for PAH extraction. Arterial blood was obtained initially for PAH, pCO_2 and pO_2. When minor adjustments of the respirator were necessary the dogs were allowed to stabilize an additional 15 min. Acetylcholine was infused at 30–50 μg/min into the right artery throughout the entire experiment. Following stabilization arterial blood gases were obtained and four 15 minutes control periods were obtained for inulin or ^{125}I iodothalmate and PAH clearances and sodium excretion. During the second period either strontium 85 or cerium 141 labelled microspheres (15 μM, 3M Company, St. Paul, MI) was given through the aortic catheter. After suspension in 10% dextran, 10×10^5 spheres containing about 10 μCi were rapidly injected and the catheter flushed with 10–15 ml of saline. Following the control period a Heidbrink Kinet-O-Meter (Lundy-Rochester) anesthesia machine with a circle CO_2 reabsorber was connected in line with the Harvard respirator. To lower the pO_2 nitrogen was added to oxygen. The addition of nitrogen allowed the arterial pO_2 to be lowered from 74 to 106 mmHg and maintained between 30 and 40 mmHg. Arterial blood gases were sampled at 5- to 10-min intervals thereafter to insure stability. Following a 30-min hypoxic equilibration period, five to seven 8-min periods were obtained for clearance of inulin or ^{125}I iodothalmate, PAH and sodium. Microspheres with a different isotope label from those used during control were injected into the aortic catheter. During recovery the anesthesia ma-

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chine was disconnected from the respirator and the dog again ventilated with room air. Following a 30-min stabilization period arterial blood gases were determined and four 10-min recovery periods obtained for inulin or ^{125}I, and PAH.

Tissue preparation for microspheres. At the conclusion of each experiment both kidneys were removed, drained of blood and frozen. A sagittal slice, 2–5 mm thick, was cut from the center of each frozen kidney. The slice was further divided into ten wedges approximately 5 mm wide by cutting perpendicular to the cortical surface. The cortical wedge was divided into four equal zones by using a preset cutting box. While still frozen the pieces were rapidly weighed on a Roller-Smith balance and placed in gamma counting tubes. The zones were numbered I (superficial) through IV (juxtamedullary) (10).

Analytic methods. Glomerular filtration (GFR) was determined by clearance of either ^{125}I iodothalmate (11) or inulin. Inulin concentrations were determined by the diphenylamine method (4). Renal plasma flow (RPF) was estimated from PAH clearance without correcting for extraction. PAH concentrations were determined by the method of Smith (12). Arterial pO_2, pCO_2 and pH were measured on an IL 113 pH-gas analyzer. Sodium was measured on an IL 143 flame photometer. Radioactivity was determined in a Nuclear Chicago two channel gamma spectrometer. Fractional blood flow distribution to the renal cortex was calculated from the equation: $P_z = qz/qt$; where P_z = percent of flow per g for a given zone uncorrected for zonal volume, qz = cpm per g of tissues in the respective zone, and qt represents the sum of cpm per g from all four cortical zones (13). The paired t test was used to determine statistical significance. All values are given as mean ± SE.

Results. Maintenance of arterial pH, pCO_2 and blood pressure during hypoxia. There were no significant differences in arterial pH between control (7.38 ± 0.01) or hypoxia at 20 min (7.38 ± .01), hypoxia at 60–90 min (7.37 ± 0.01) or during recovery (7.36 ± .01). The pCO_2 also remained unchanged during these periods (36.5 ± 1.6, 36.7 ± 1.6, 35.8 ± 1.8, 34.8 ± 1.9). Thus, extracellular acid base conditions were maintained constant throughout the experiment. Mean blood pressure increased from 111 ± 2 mmHg to 119 ± 5 mmHg during hypoxia ($P < .05$) and returned to control level during recovery (112 ± 5 mmHg).

The effect of hypoxia on GFR and C_{PAH}. The effect of hypoxia on GFR and C_{PAH} in acetylcholine perfused and nonperfused kidneys is shown in Table I. The GFR of non acetylcholine perfused kidneys fell 33 ± 7% ($P < .001$) during hypoxia. Although the percent change varied, the GFR decreased in each kidney. Restoration of normal oxygen tension caused the GFR to increase in all nonperfused kidneys and the GFR for the entire group had returned to the control level. C_{PAH} also decreased in all nonperfused kidneys. The mean decrease of 38 ± 6% was significant ($P < .005$).

In acetylcholine perfused kidneys GFR did not change during hypoxia. The difference in response of GFR to hypoxia between perfused and nonperfused kidneys is significant ($P < .005$) showing that acetylcholine prevents the hypoxia induced decrease in GFR. The mean 6% decrease in C_{PAH} of perfused kidneys during hypoxia was not statistically significant. The difference in C_{PAH} between perfused and nonperfused kidneys is significant ($P < .005$) so it appears that acetylcholine prevents a decrease in C_{PAH} during hypoxia.

In four additional dogs hypoxia was induced without infusing acetylcholine and PAH extraction determined. During hypoxia GFR fell 42% ($P < .005$) while C_{PAH} fell 35% ($P < .025$). The renal extraction of PAH was 0.76 ± .07 in controls, 0.79 ± .09 during hypoxia, and 0.76 ± .04 in recovery. These differences were not significant.

Effect of hypoxia on cortical blood flow. Fractional blood flow to the renal cortex determined by microsphere distribution is shown in Fig. 1. The fraction of blood flow to inner and outer cortical zones was identical during control and hypoxia. Therefore, the increased vascular resistance caused by hypoxia is evenly distributed throughout the kidney demonstrating that the decrease in whole kidney GFR and RPF are not the result of a redistribution of cortical blood flow. Cortical blood flow distribution in acetylcholine perfused kidneys likewise was un-

TABLE I. EFFECT OF HYPOXIA ON GFR AND RPF IN DOGS WITH UNILATERAL RENAL ACETYLCHOLINE PERFUSION.

	GFR (ml/min)						C_{PAH} (ml/min)					
	No Ac			Ac			No Ac			Ac		
	C	H	R	C	H	R	C	H	R	C	H	R
	50.3	40.8	48.8	50.6	46.4	44.7	154.4	135.6	136.3	187.3	174.8	173.8
	24.1	21.7	23.1	35.8	46.9	24.4	70.7	57.6	43.4	101.6	104.8	91.6
	45.8	28.9	38.9	18.1	16.7	—	95.3	59.2	63.1	64.6	58.9	—
	23.6	13.9	20.8	18.2	17.2	15.9	63.6	59.9	91.9	79.1	86.3	109.9
	22.1	19.1	25.3	26.8	24.0	25.7	117.6	99.0	96.1	141.5	134.0	119.7
	41.1	21.5	32.7	36.0	34.1	31.5	130.4	73.8	92.0	130.6	129.6	101.0
	25.1	17.2	21.9	28.5	21.7	21.2	91.2	61.4	66.8	122.3	92.7	78.9
	29.9	10.3	48.1	33.8	36.1	39.5	155.6	62.3	175.6	148.9	155.3	172.0
$\bar{X}$	32.8	21.7*	32.5†	31.0	30.4	29.0	109.9	76.1*	95.7	122.0	117.1	121.0
SEM	4.26	3.60	4.38	4.03	4.64	4.19	13.42	10.48	16.13	15.01	14.53	15.47

GFR, glomerular filtration rate; C, control; H, hypoxia; R, recovery; Ac, acetylcholine perfused kidney; No Ac, kidney without acetylcholine perfusion.
* Comparing C to H; $P < .01$; † comparing H to R; $P < .025$.

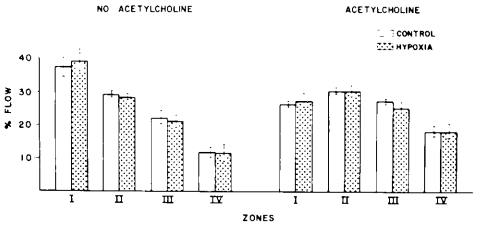

FIG. 1. Fractional blood flow to renal cortex in acetylcholine perfused and nonperfused kidneys. Results represent mean fractional flow ± SEM to each cortical zone.

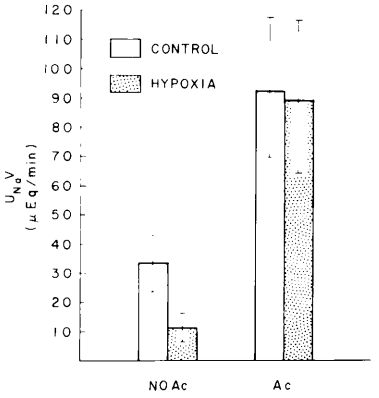

FIG. 2. Effect of hypoxia on sodium excretion. Sodium excretion ($U_{Na}V$) from non acetylcholine perfused kidneys (no Ac) is compared to acetylcholine perfused kidneys (Ac) during hypoxia. The brackets represent ±SEM.

changed by hypoxia. These microsphere data indicate that the maintenance of GFR and RPF with acetylcholine is a result of general renal vasodilatation rather than a redistribution of renal blood flow by the drug.

The effect of hypoxia on sodium excretion. Sodium excretion decreased in all the nonperfused kidneys. As shown in Fig. 2 mean sodium excretion in nonperfused kidneys decreased 65 ± 9% from control ($P < .001$). By contrast the mean 8 ± 10% decrease in sodium excretion of perfused kidneys was not significant. Thus, the decrease in sodium excretion caused by hypoxia is prevented by vasodilatation with acetylcholine.

Discussion. Although many studies in dogs have examined the effect of hypoxia on renal function, much of the data are contradictory. Earlier dog studies demonstrated that moderate hypoxia results in either no change (1, 3) or a decrease in GFR (2). Moreover, RPF was reported to be either increased (1), decreased (2, 4) or unchanged (3) and changes in RPF did not always parallel changes in GFR. Sodium excretion also varied independently from changes in RPF and GFR and was found to be either decreased (3) or unchanged by hypoxia (2). Alterations in extracellular acid base conditions may at least partially account for the disparate results since pH was neither monitored nor controlled in the studies cited. In most of the previous studies hypoxia was associated with hyperventilation which may have caused a respiratory alkalosis. Alkalosis decreases renal vascular resistance (8) and causes a sodium diuresis (14). There are two studies, however, in which the pCO_2 was maintained constant. In the study of Cosgrove et al (15) most of the animals developed a progressive and unexplained metabolic acidosis. Acidosis

can either increase (6, 7, 9) or decrease vascular resistance (8) depending on the degree of acidosis. Kalyonides (3) maintained pCO_2 and pH constant while decreasing the pO_2 from 400 to 44 mmHg in dogs and was unable to detect any change in GFR or RPF. However, raising the arterial oxygen to a level of only 160 mmHg significantly decreases RPF (16). If the initial GFR and RPF in Kalyonides' studies were already reduced by the high level of oxygen, altered renal function induced by acute hypoxia might be undetectable. The present study clearly shows that when acid-base conditions and control oxygen tension are maintained hypoxia reduces GFR, C_{PAH} and sodium excretion.

Several mechanisms could be responsible for these hypoxia induced effects. First, hypoxia might decrease cardiac output which could then lead to a decrease in both RPF and GFR. Although cardiac output was not measured in this study blood pressure remained constant. Also, it has been shown that hypoxia actually augments cardiac output in dogs made acutely hypoxic to the same extent employed in this study (5, 17). It seems unlikely, therefore, that cardiac output was altered. Second, hypoxia might injure renal tubules resulting in back diffusion of PAH and inulin falsely lowering the calculated GFR and RPF. In various models of acute renal failure PAH (18) and inulin (19) have been shown to diffuse from the renal tubule into the renal venous blood resulting in a decrease in calculated RPF and GFR. However, in the four dogs examined in this study PAH extraction was not altered by hypoxia. Had significant back diffusion of PAH accounted for the decrease in calculated RPF the extraction ratio should have decreased. In addition, maintenance of PAH and inulin clearances during hypoxia in the acetylcholine perfused kidney at a time when these clearances had decreased in the non perfused contralateral kidney also argues against a hypoxic alteration of renal tubular cell integrity. Third, redistribution of cortical blood flow during hypoxia could decrease GFR and RPF. A functional decrease in RPF and GFR has been associated with a redistribution of blood away from the outer renal cortex (20). Redistribution of cortical blood flow, however, cannot account for the hypoxia induced decrease in hemodynamic function found in this study since fractional flow distribution in the cortex was not altered. Finally, a diffuse increase in renal vascular resistance either functionally or as a result of direct ischemic injury with swelling or spasm of the renal vasculature could explain the decrease in RPF and GFR.

The decrease in RPF when considered with the microsphere data demonstrates that hypoxia diffusely increases renal vascular resistance. Since filtration fraction was unchanged, resistance must have increased in both afferent and efferent arterioles. The return towards control levels of RPF and GFR during recovery demonstrates the reversibility of this increased resistance but cannot truly distinguish between a functional increase in resistance and a direct toxic effect on the renal vasculature resulting from ischemia and cell swelling (21, 22). However, since renal hemodynamic function is maintained during vasodilatation with acetylcholine a direct toxic effect of hypoxia seems unlikely.

The large decrease in sodium excretion seen during hypoxia can be accounted for by the decreases in both renal perfusion and GFR (4). Whether hypoxia exerts a separate effect on renal tubular sodium reabsorption such as altering single nephron filtration fraction (10) was not examined in this study. However, since sodium excretion is maintained with acetylcholine perfusion, it seems unlikely that hypoxia has any direct effect on sodium reabsorption separate from its effect on renal perfusion.

Summary. Previous studies of hypoxia induced alterations in renal function have yielded conflicting results. Uncontrolled acid base conditions in most studies may account for this scatter. To eliminate acid-base effects hypoxia was induced in dogs while arterial pCO_2 and pH were maintained constant for 60–90 min. To test whether the effects of hypoxia were mediated by vasoconstriction or ischemic injury, acetylcholine was infused unilaterally into one renal artery. During hypoxia the GFR and RPF of the kidney not receiving acetylcholine decreased significantly. In the kidney perfused with acetylcholine, however, GFR and RPF did not change. Sodium excretion fell in nonperfused but did not change significantly in acetylcholine perfused kidneys. Using the radiomicrosphere

method, fractional distribution of renal cortical blood flow was found to be unaffected by hypoxia. The data demonstrate that acute hypoxia reversibly decreases GFR and RPF by functionally increasing renal vascular resistance uniformly throughout the kidney and that these changes were associated with a decrease in sodium excretion.

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