Kidney International, Vol. 14 (1978), pp. 58–67

Renal function and intrarenal hemodynamics in acutely hypoxic and hypercapnic rats

BRIGITT ZILLIG, GEORG SCHULER, and BRUNO TRUNIGER

Department of Medicine, Kantonsspital, Lucerne, Switzerland

Renal function and intrarenal hemodynamics in acutely hypoxic and hypercapnic rats. On the basis of microsphere distribution, inert gas washout, and standard clearance data, the effects of acute hypoxia and hypercapnia on the kidney were studied in anesthetized, mechanically ventilated rats. *Moderate hypoxia* (mean Po_2, 48 mm Hg) did not significantly change diuresis, GFR, and tubular sodium rejection. Due to a decrease in renal vascular resistance (R) from 40.1 to 31.8 mm Hg·ml^{-1}·min, mean renal blood flow stayed constant in spite of a significant drop in mean arterial blood pressure. Hypoxic changes in R were not accompanied by significant changes in intrarenal distribution of blood flow (IDBF). In *severe hypoxia* (Po_2 < 45 mm Hg) with oliguria and marked arterial hypotension, R was the lowest of all groups (28.8 mm Hg·ml^{-1}·min). *Hypercapnia* did not significantly change the renal excretory parameters, although an increase in R (without change in IDBF), together with a decrease in MAP caused a marked drop in mean renal blood flow. From these studies we conclude: *1)* in the anesthetized rat, acute hypoxia causes significant changes in intrarenal hemodynamics without changes in excretory function, *2)* hypoxic renal vasodilatation persists even in severe hypotension with oliguria and anuria, *3)* in acute hypoxia and hypercapnia, changes in renal blood flow and renal vascular resistance are not accompanied by significant changes in IDBF.

Fonction rénale et hémodynamique intrarénale chez des rats soumis à une hypoxie et une hypercapnie aiguës. Les effets de l'hypoxie et de l'hypercapnie aiguës sur le rein ont été étudiés chez des rats, anesthésiés et ventilés mécaniquement, au moyen de la distribution de microsphères, des courbes de lavage des gaz inertes et des clearances. L'hypoxie modérée (Po_2 moyenne de 48 mm Hg) ne modifie pas significativement la diurèse, la filtration glomérulaire et l'excrétion du sodium. Du fait d'une diminution de la résistance vasculaire (R) de 40,1 à 31,8 mm Hg·ml^{-1}·min le débit sanguin moyen n'a pas varié malgré une chute significative de la pression artérielle moyenne. Ces modifications hypoxiques de R n'ont été accompagnées par des modifications significatives de la distribution du débit sanguin rénal (IDBF). Dans l'hypoxie sévère (Po_2 < 45 mm Hg) avec oligurie et hypotension artérielle importante, R était le plus bas de tous les groupes (28,8 mm Hg·ml^{-1}·min). L'hypercapnie ne modifie pas significativement les paramètres d'excrétion, bien qu'une augmentation de R (sans modification d'IDBF) en même temps qu'une diminution de MAP détermine une chute du débit sanguin moyen. De ces résultats nous concluons que: *1)* chez le rat anesthésié l'hypoxie aigue détermine une modification significative de l'hémodynamique intrarénale sans modification de la fonction excrétoire, *2)* la vasodilatation hypoxique persiste dans l'hypotension sévère avec oligurie et anurie, *3)* dans l'hypoxie et l'hypercapnie aiguës les modifications du débit sanguin rénal et des résistances vasculaires ne sont pas accompagnées par des modifications significatives d'IDBF.

Received for publication October 19, 1976
and in revised form January 31, 1978.

0085-2538/78/0014-0058 $02.00

Controversy exists concerning the effects of acute respiratory failure on renal function. Both increased and decreased glomerular filtration rate, natriuresis and diuresis have been reported, depending apparently on the animal species used, on the mode of hypoxygenation, the severity and duration of hypoxia, and, perhaps, on the use and technique of anesthesia (for literature survey, see Ref. 1 and 2). Similarly, rather inconsistent changes in renal hemodynamics (renal blood flow, renal vascular resistance) have been observed in human as well as in animal studies [1–5]. There is particularly scant information, so far, on changes in intrarenal hemodynamics—intrarenal and intracortical distribution of blood flow and local intrarenal blood flow rates—induced by acute hypoxia or hypercapnia [5].

The present studies, designed primarily to fill this gap, indicate that although a massive renal vasodilatation and drop in renal vascular resistance occurred in response to hypoxia, there was no significant change in the intrarenal distribution of blood flow, as shown by microsphere distribution analysis and inert gas washout data. Acute hypercapnia increases renal vascular resistance, again without changing the intrarenal distribution of blood flow.

Methods

Experiments were performed with male Wistar rats weighing between 200 and 300 g. The animals were fed a standard laboratory chow. Food was withheld 12 hr prior to experiments but drinking water was accessible. Animals were placed on a heated operating table and were anesthetized with

pentobarbital (Nembutal®, 6 to 8 mg/100 g, i.p.). The minimum standard procedure included tracheotomy and catheterization of the left external jugular vein and the left carotid artery with polyethylene tubing (PP 25; O.D., 0.8 mm; I.D., 0.4 mm). Saline was infused through the carotid artery catheter at constant rates (1 ml/hr) with a perfusor (Braun, Mark IV). In preparing the carotid artery, care was taken not to damage the vagal nerve and its branches, and the contralateral carotid artery was always left untouched. Urine was collected through an indwelling polyethylene bladder catheter (placed via a small suprapubic incision), and urinary flow rates were measured by timed collections into a precalibrated piece of polyethylene tubing (PP 90; O.D., 1.27; I.D., 0.86 mm).

The rats were either allowed to breathe spontaneously (series A) or they were artificially ventilated (Schuler ventilator, Mark II, Braun, Melsungen; airflow limited, no positive end expiratory pressure). Relaxation was induced and sustained by repeated doses of pancuronium bromide (Pavulon®, single doses of 0.4 to 0.6 mg; all series). On the basis of numerous experiments, the pentobarbital dosage was adjusted for an equal and suitable depth and duration, so that the animals did not experience pain (no reflex withdrawal of hind paw upon pinching the foot pad and toes). The following gas mixtures were used for ventilation: mixture I: 21% oxygen, 2% carbon dioxide, 77% nitrogen; mixture II: 15% oxygen, 2% carbon dioxide, 83% nitrogen; mixture III: 10% oxygen, 2% carbon dioxide, 88% nitrogen; mixture IV: 21% oxygen, 10% carbon dioxide, 69% nitrogen.

Due to the complexity of the experimental protocol and to avoid mutual interference of the various methods involving radioactively labeled indicator substances, the experiments had to be done using different series of animals.

In a first pilot series (N1) of seven rats, the stability of the rat preparation was tested: the animals were ventilated with the same gas mixture (mixture I) during 120 min. Blood gas and renal excretory parameters were checked at times identical to the series A, B, and C.

Series A: Effects of mechanical ventilation (10 rats). Arterial blood gas and renal excretory parameters (urine flow rate, glomerular filtration rate (GFR), and renal sodium rejection), as well as mean arterial blood pressure (MAP) were measured under conditions of spontaneous breathing and mechanical ventilation with gas mixture I.

Series B: Effects of acute hypoxia (10 rats). The same parameters were checked as in series A, under conditions of normoxic (gas mixture I) versus hypoxic (mixture II and III) ventilation.

Series C: Effects of acute hypercapnia (13 rats). Same protocol was followed as in series B: ventilation with gas mixture I, followed by mixture IV.

Series D: Effects of acute hypoxia on hemodynamic parameters as measured by the inert gas washout technique (11 rats). Under conditions of normoxic (gas mixture I) vs. hypoxic ventilation (gas mixture II and III), mean renal blood flow ($\bar{F}$; ml/min·100 g), kidney weight (KW), and MAP were measured and total renal vascular resistance (R; mm Hg·ml^{-1}·min) was calculated according to the formula $R = MAP/\bar{F} \cdot KW$.

Series E: Effects of acute hypercapnia on hemodynamic parameters (12 rats). Same protocol was followed as in series D: ventilation with gas mixture I, followed by gas mixture IV.

Two additional *pilot series* (N2 and N3) were used to evaluate, in the rat, the microsphere distribution method as a means of measuring the intracortical distribution of blood flow. *Series N2:* in 10 animals, under conditions of artificial ventilation with 21% oxygen, the effect on renal, excretory, function of a single injection of some 440'000 to 660'000 unlabeled microspheres (diameter, 15 ± 5 μ, as indicated by the manufacturer) was studied (urine flow rate, GFR, sodium excretion and sodium rejection). *Series N3:* in 14 rats, the trapping site, the size of the trapped spheres, and the venous outflow of microspheres were studied, following the procedure outlined elsewhere [6].

Series F: Reproducibility of the microsphere distribution (20 rats). This was studied while the rats were continuously ventilated with gas mixture I. At an interval of 30 min, two injections of differently labeled microspheres were made.

Series G: Effect of acute hypoxia on intracortical distribution of microspheres (14 rats). The intracortical distribution of blood flow was studied under conditions of artificial ventilation with 21% oxygen vs. 15% and 10% oxygen (gas mixture I vs. II and III).

Series H: Effect of acute hypercapnia on microsphere distribution (16 rats). Same protocol was followed as in series G: ventilation with gas mixture I (2% carbon dioxide), followed by gas mixture IV (10% carbon dioxide).

MAP was monitored by mercury manometry via the carotid artery or the abdominal aortic catheter (catheter tip at or just below the ostium of the left renal artery). Arterial blood samples taken from either one of these catheters were analyzed on a blood gas analyzer (Instrumentation Laboratories, Model 213/227). Sodium concentrations in urine (U)

and plasma (P) samples were measured by flame photometry. Sodium rejection (expressed as % of filtered load) was calculated from the U/P ratios for sodium and ^{51}Cr-EDTA. GFR was determined on the basis of the ^{51}Cr-EDTA clearance rate. (^{51}Cr-EDTA was obtained from EIR, Würenlingen, Switzerland; specific activity, 0.76 to 1.5 mCi/mg.) After a loading dose of 50 μCi, a sustaining infusion of 20 μCi/hr was given. After an equilibration period of 30 to 45 min, two clearance periods of 15 to 30 min's duration followed for each of the experimental conditions. Urine and blood samples were counted in an autogamma-spectrometer (Packard 5200), and clearance calculations were made in standard fashion [7].

Mean renal blood flow. This was determined on the basis of the initial slope of the ^{133}Xenon washout curve [8]. The rats were prepared for the inert gas washout studies as described previously [9]. The washout of 100 to 200 μCi of ^{133}Xe from the kidney was monitored during 10 min, and the analysis of the washout curve followed the standard procedure [10]. Complete analysis of the washout curves resulted in three single exponential components. CP-I, the component representing the compartment with the most rapid local blood flow rate, closely reflects cortical blood flow [8, 9, 11]. Two measurements were made for each experimental condition.

Microsphere distribution studies. These used radioactively labeled microspheres (strontium 85 and cerium 141) of 15 ± 5 μ in diameter (3M Company). The spheres were resuspended in rat plasma, sonicated, and vigorously agitated just prior to the injection into the left ventricle via the carotid catheter. The supernatant of each batch of microspheres was checked for radioactivity. No significant amount of gamma radiation was found. Each animal received two consecutive injections within 30 min, either without any change in the experimental conditions (reproducibility testing) or before and during hypoxia or hypercapnia. In the latter two series, the first injection was made at the end of the 30-min equilibration period, during which the animal was artificially ventilated with 21% oxygen. The second one followed after 30 min of hypoxic ventilation. After the second microsphere injection, the animal was killed, and both kidneys were removed, weighed, and placed in 10% neutral formalin for 48 hr. Eight control kidneys were prepared for microscopic examination, cut into 8-μ and 25-μ-thick sections, stained with hematoxylin and eosin dye, and examined for microsphere trapping site and size of the trapped spheres. All other kidneys were freed from hilar and perirenal fat, and two coronal sections of the midportion of the kidney, 1.5- to 3.0-mm-thick, were cut. The medullary region (outer and inner medulla) was separated from the cortex with sharp U-shaped blades, and the remaining kidney cortex was divided into two regions along a line judged by eye to be midcortical thickness (OC, outer cortex; IC, inner cortex). In retrospect, calculations based on kidney weight and the weight of the outer and inner cortical tissue samples indicate a mean thickness of 1.4 mm for the inner cortex and 1.1 mm for the outer cortex. The outer and inner cortical tissue samples were pooled separately, weighed, and counted in an autogamma-spectrometer (Packard 5200). Each coronal sectioning was done separately, and the average of both sections was used to calculate the data included in Table 4.

With the assumption that the microsphere distribution in the relatively thin coronal section was representative for the whole kidney, the radioactivity of the outer cortical tissue divided by the radioactivity of the whole coronal slice (OC/S) reflects the intrarenal distribution of blood flow. Since only occasional and negligible amounts of radioactivity were found in the outer medullary tissue (probably due to the problem of a clear-cut separation between cortex and medulla), the OC/S ratio equals the OC/C ratio, the outer cortical fraction of cortical blood flow. In order to eliminate the problem of dissection errors, each animal was used as its own control, and changes in outer cortical flow fraction are given as the ratio (Q) of OC/C under control conditions (OC_1/C_1) and under experimental conditions (OC_2/C_2). With this procedure, a ratio $Q < 1$ will indicate an increase, and a $Q > 1$ will indicate a decrease in outer cortical flow fraction as compared to control conditions.

Statistical analysis of the data was performed using Student's *t* test for paired data and independent samples.

Results

Acute effects of artificial ventilation, hypoxia, and hypercapnia on blood gas parameters, MAP, and renal excretory function (Table 1). It is important to realize that a majority of anesthetized, nonventilated rats are distinctly hypoxic as compared with accepted standard values (Po_2, 91.8 ± (SD) 3.5 mm Hg; HbO_2, 91.3 ± (SD) 3.2%; Pco_2, 38.0 ± (SD) 3.1 mm Hg; pH, 7.40 ± (SD) 0.015 [12, 13]). While mechanical ventilation of the anesthetized and relaxed rat was allowed to increase and normalize the Po_2 values, it simultaneously induced a marked hypocapnia. The problem was solved by adding 2% carbon dioxide to the gas mixtures, resulting, in the case of gas mixture I, in blood gas analyses adequate

Table 1. Acute effects of artificial ventilation, hypoxia, and hypercapnia on blood gas parameters, mean arterial blood pressure (MAP), and renal excretory function

		Po_2 *mm Hg*	HbO_2 %	Pco_2 *mm Hg*	pH	Std. Bic. *mEq/liter*	MAP *mm Hg*	GFR[a] *ml/min · kg*	$\dot{V}_u$[b] *µl/min*	$Na^+{}_{RF}$[c] %
					Series A: Spontaneous breathing (1) vs. artificial ventilation (2) (N = 10)					
1)	$\bar{x}$	71.7	95.0	40.9	7.43	26.2	126.0	8.92	9.48	0.30
	SD	5.8	1.1	4.5	0.03	3.8	8.8	2.50	4.22	0.25
2)	$\bar{x}$	98.0	96.6	43.1	7.39	19.8	125.5	7.34	6.98	0.51
	SD	14.1	2.2	6.2	0.04	2.3	14.4	2.93	6.62	0.49
	P[d]	—	—	—	—	—	NS	NS	NS	NS
					Series B (hypoxia): Artificial ventilation with 21% O_2 (1) vs. 15% O_2 (2) (N = 10)					
1)	$\bar{x}$	97.1	97.0	47.0	7.36	25.9	137.9	7.91	4.83	0.65
	SD	9.5	0.9	6.1	0.07	4.3	17.0	1.61	2.60	0.42
2)	$\bar{x}$	47.9	80.6	47.5	7.33	24.0	100.4	8.96	5.44	0.61
	SD	7.4	8.0	4.2	0.07	3.8	24.9	3.62	5.84	0.59
	P[d]	—	—	—	—	—	< 0.001	NS	NS	NS
					Series C (hypercapnia): Artificial ventilation with 2% CO_2 (1) vs. 10% CO_2 (2) (N = 13)					
1)	$\bar{x}$	89.3	96.4	47.8	7.37	25.7	138.2	10.79	2.44	0.97
	SD	6.7	0.7	3.6	0.03	2.3	8.5	2.51	0.74	0.45
2)	$\bar{x}$	100.3	95.2	98.1	7.14	26.2	125.9	11.01	3.53	2.33
	SD	10.2	1.2	9.2	0.03	3.5	10.1	2.32	0.97	1.53
	P[d]	—	—	—	—	—	< 0.001	NS	< 0.001	< 0.001

[a] ^{51}Cr-EDTA clearance.
[b] Urine flow rate.
[c] Sodium rejection fraction (% of filtered sodium load).
[d] Significance of differences between groups (NS = not significant).

to serve as baseline conditions for the hypoxia and hypercapnia studies. With considerable individual variations, the hypoxic gas mixtures induced a drop in Po_2 and HbO_2 of the arterial blood with constant Pco_2 values, while mixture IV (10% carbon dioxide) caused a reproducible severe hypercapnia without significantly changing Po_2 and HbO_2 values.

The pilot studies (series N1) revealed a satisfactory stability of the rat preparation: no significant changes in blood gas parameters, MAP, GFR, and diuresis during 120 min of normoxic ventilation. Whether a small, but significant ($P < 0.025$) increase in sodium rejection from 0.47% (SD, 0.49) to 1.03% (SD, 1.01) was due to the ventilation procedure or to the peripheral infusion of saline has not been further tested.

In a majority of animals, MAP dropped considerably upon ventilation with the hypoxic gas mixtures. While this initial hypotension was reduced by extracellular volume replacement, it turned out that, independent of volume replacement, MAP stabilized at essentially the same level within 30 min. Therefore, volume substitution was abandoned, and all animals received the same infusion rate of one milliliter of saline per hour. As shown in series A, artificial ventilation *per se* did not significantly alter MAP or renal excretory parameters, such as GFR, diuresis, or fractional sodium excretion.

In spite of a significant drop in MAP, ventilation with 15% oxygen did not change GFR, diuresis, or renal sodium rejection. This is in sharp contrast to an earlier series of similar experiments in which volume substitution induced a brisk diuresis and natriuresis despite a comparable degree of arterial hypotension. Due to the circulatory instability of the animals, no determinations of GFR or fractional sodium excretion were made in the 10% oxygen group.

As shown in Table 1, series C, acute hypercapnia caused a small but significant increase in diuresis and renal sodium rejection (RF). Expressed as a ratio ($RF_{control}/RF_{exp.}$), the increase in RF in series C is not significantly different from that observed in pilot series N1 ($P > 0.1$). The same applies for the increase in diuresis.

Acute effects of hypoxia and hypercapnia on intrarenal hemodynamics: a) Xenon studies (Table 2). For reasons to be discussed in the following section, baseline values for mean arterial blood pressure (MAP) and total renal vascular resistance (R) are higher, and mean renal blood flow ($\bar{F}$) is significantly lower than those reported in our previous studies ($\Delta \bar{x} = 13$ mm Hg, $P < 0.01$ for MAP; $\Delta \bar{x} = 15$ mm Hg $\cdot ml^{-1} \cdot$ min, $P < 0.005$ for R; $\Delta \bar{x} = -62$ ml/min $\cdot$ 100 g, $P < 0.005$ for $\bar{F}$ as compared with Ref. 14). The CP-I flow fraction agrees well with the previously reported data. Artificial ventilation with 15% oxygen

Table 2. Effects of hypoxia and hypercapnia on intrarenal hemodynamics (^{133}Xenon studies)

		MAP *mm Hg*	$\bar{F}$[a] *ml/min·100 g*	R[b] *mm Hg·ml^{-1}·min*	CP-I[c] %	F_I[d]	P_{O_2} *mm Hg*	P_{CO_2} *mm Hg*
		Series D (hypoxia): Artificial ventilation with 21% O_2 (1) vs. 15% O_2 (2) (N = 11)						
1)	$\bar{x}$	125.0	290.9	40.1	85.3	348.6	102.6	48.5
	SD	15.3	46.2	10.6	2.6	67.6	15.3	10.3
2)	$\bar{x}$	92.3	275.4	31.8	84.9	329.4	57.0	48.6
	SD	18.6	69.9	9.1	3.3	62.3	10.3	8.2
	P[e]	<0.001	NS	<0.001	NS	NS	<0.001	NS
		Series D' (hypoxia): Hypotensive animals only (MAP < 100 mm Hg) (N = 6)						
1)	$\bar{x}$	116.7	291.3	41.3	84.5	326.6	100.8	49.0
	SD	14.4	60.6	11.2	2.9	59.9	13.4	9.7
2)	$\bar{x}$	77.5	252.4	32.4	83.4	311.8	53.3	49.5
	SD	9.4	67.0	8.4	3.8	62.5	12.1	10.4
	P[e]	<0.001	NS	<0.01	NS	NS	<0.001	NS
		Series E (hypercapnia): Artificial ventilation with 2% CO_2 (1) vs. 10% CO_2 (2) (N = 12)						
1)	$\bar{x}$	120.6	277.1	34.2	85.1	323.0	99.6	48.2
	SD	13.5	70.8	7.8	4.3	68.7	12.6	8.9
2)	$\bar{x}$	114.2	228.9	40.2	83.4	264.1	109.7	105.9
	SD	11.8	69.5	11.1	5.1	73.3	13.8	9.6
	P[e]	<0.01	<0.001	<0.005	NS	<0.001	NS	<0.001

[a] Mean renal blood flow.
[b] Renal vascular resistance.
[c] Flow fraction distributed to CP-I (most rapid local blood flow rate).
[d] Local blood flow rate corresponding to CP-I.
[e] Significance of difference between control conditions and experimental conditions (paired *t* statistics; NS = not significant).

caused a significant drop in MAP without concomitant changes in $\bar{F}$. From these data, including kidney weight, a significant reduction in renal vascular resistance is calculated. These changes were even more marked in rats ventilated with a gas mixture containing 10% oxygen (P_{O_2}, 45.8 ± (SD) 12.0 mm Hg; MAP, 68.8 ± (SD) 22.3 mm Hg; $\bar{F}$, 173.8 ± (SD) 44.1 ml/min ·100 g; R, 33.4 ± (SD) 12.8 mm Hg·ml^{-1}·min). In this group, a significant drop in mean renal blood flow was not prevented by a further decrease in renal vascular resistance. A small reduction in CP-I flow fraction in both hypoxic groups did not attain statistical significance.

Since the circulatory response to hypoxia was rather heterogeneous, as indicated by the standard deviation of MAP, the animals of series D were regrouped according to their change in MAP. An arbitrary critical value of 100 mm Hg was chosen (series D', Table 2). Again, a decrease in renal vascular resistance was observed, not significantly different from the animals with a MAP > 100 mm Hg during hypoxia. None of the groups revealed a significant decrease in CP-I flow fraction. Other regrouping criteria, such as the severity of hypoxia as judged by blood gas analysis, or hypotension as defined as a pressure drop of > 20 mm Hg, did not result in additional significant differences in intrarenal hemodynamics.

In our entire series of experiments, there were six animals responding to severe hypoxia with marked oliguria and anuria (mean urinary flow rate, 0.42 ± (SD) 0.47 μl/min·100 g). Mean arterial blood pressure (64.7 mm Hg) and total renal vascular resistance (28.8 mm Hg·ml^{-1}·min) were among the lowest of all groups.

Acute hypercapnia (series E, Table 2) caused a significant decrease in mean renal blood flow which was mainly due to a marked increase in R by almost 20% of the control value. Again this change was not accompanied by a change in the flow fraction distributed to CP-I of the washout curve.

b) Radioactive microsphere distribution data (series F, G, H, Tables 3 and 4). Prior to applying the microsphere technique to the hypoxia studies, a number of methodological problems had to be solved. *Microscopic examination* of coronal histological sections of the kidneys injected with unlabeled 15-μ microspheres led to the distribution data given in Table 3. It is obvious that a majority of over 90% of the spheres became lodged in the glomerular capillaries and afferent arterioles, a minority being trapped in interlobular arteries. Impaction of microspheres within intrarenal arteries occurred in a negligible percentage of injected spheres. These microscopic findings are in good agreement with the data obtained by counting the radioactivity of the tissue

Table 3. Microscopic anatomical distribution of microspheres (MS) in four kidneys of two rats[a]

Localization	No. of MS	% of total MS counted	Mean diameter of MS ($\bar{x}$ ± SD) μ	MS/glomerulum
Total MS counted	6,880	100.0	12.77 ± 1.14	2.11
outer cortex	5,624	81.8	12.85 ± 1.16	2.30
inner cortex	1,256	18.2	12.69 ± 1.11	1.53
medulla	—	—	—	—
MS lodged in				
glomeruli	5,664	82.3	12.76 ± 1.14	—
afferent arterioles	697	10.1	12.61 ± 1.02	—
interlobular arteries	399	5.8	12.85 ± 1.07	—
arcuate and interlobar arteries	—	—	—	—
other[b]	120	1.8	—	—

[a] 25-μ sections were used. Total number of glomeruli screened was 3,266 (outer cortex, 2,445; inner cortex, 821).
[b] MS not within vascular structures (tubular lumen, interstitium, etc.).

samples (Table 4): under the conditions of this study almost 90% of the cortical radioactivity was located in the outer cortex, while 82% of the spheres were located microscopically in outer cortical structures. The size of the spheres trapped in the outer cortex did not significantly differ from that of the beads located in the inner cortical structures (Table 3).

In 10 animals of the pilot series, the injection of unlabeled microspheres did not appreciably change urinary flow rates and sodium excretion. A small decrease in GFR from 8.8 to 7.7 ml/min·100 g and a drop in MAP from 127 to 120 mm Hg did not attain statistical significance. Since the number of microspheres used in the hypoxia and hypercapnia experiments was smaller, ranging from 100'000 to 400'000 per injection, it seems reasonable to conclude that renal function was not significantly influenced by the spheres. The *reproducibility studies* of microsphere distribution in two consecutive injections (series F, Table 4) indicate a small but statistically significant drop in outer cortical flow fraction (OC/C) after the first microsphere injection ($P < 0.001$). The resulting ratio of 1.07, SD 0.07, for $Q_{control/repeat}$ is therefore taken as the reference point for the experiments of series G and H.

In spite of the marked changes in total renal vascular resistance, *hypoxia* did not change the intracortical distribution of microspheres when all hypoxic animals were lumped irrespectively of changes in MAP. Again, regrouping the animals of the whole

Table 4. Effects of hypoxia and hypercapnia on intracortical distribution of microspheres

	Control conditions[a] OC_1/C_1	Experimental conditions OC_2/C_2	$P_{control/exp}$	$Q_{control\ exp}$[d]
	Series F: Reproducibility studies (artificial ventilation with 21% O_2) (N^b = 40)			
$\bar{x}$	0.900	0.853	<0.001	1.07
SD	0.058	0.080		0.07
	Series G: Hypoxia (artificial ventilation with 15% O_2) (N = 28)			
$\bar{x}$	0.879	0.852	<0.001	1.04
SD	0.041	0.054		0.05
$P_{F/G}$[c]				NS
	Series G': Hypoxia (hypotensive animals only; MAP < 100 mm Hg) (N = 10)			
$\bar{x}$	0.872	0.815	<0.0025	1.07
SD	0.032	0.042		0.05
$P_{G/G'}$[c]				NS
	Series H: Hypercapnia (artificial ventilation with 10% CO_2) (N = 32)			
$\bar{x}$	0.880	0.874	NS	1.01
SD	0.027	0.046		0.07
$P_{F/H}$[c]				0.05

[a] Control conditions were mechanical ventilation with 21% oxygen, 2% carbon dioxide. OC/C = outer cortical fraction of total cortical radioactivity.
[b] N = number of measurements (= kidneys); $N/2$ = number of animals.
[c] P = significance of difference from reproducibility group (t statistic for two means). NS = significant.
[d] Changes in outer cortical flow fractions (see text).

series according to changes in MAP did not reveal any additional changes in microsphere distribution (series G', Table 4).

Acute hypercapnia (series H) did not change the intracortical distribution of microspheres. Although, as compared with the reproducibility series, this may be taken as indicating a small redistribution towards the outer cortex, we feel that this conclusion is not justified on the basis of the data.

Discussion

Our observations indicate *1)* that due to a marked renal vasodilatation and decrease in renal vascular resistance, mean renal blood flow is maintained in moderate hypoxia (15% oxygen ventilation gas mixture), despite a significant decrease in MAP. *2)* In severe hypoxia (10% oxygen), this decrease in renal vascular resistance does not prevent a decrease in mean renal blood flow secondary to a further drop in systemic blood pressure. *3)* Acute hypoxia induces its changes in renal hemodynamics without affecting the intrarenal distribution of blood flow and without influencing GFR, diuresis, or renal sodium rejection. *4)* Acute hypercapnia increases renal vascular resistance and decreases renal blood flow, again without changing the intrarenal or intracortical distribution of blood flow.

In this study, the analysis of intrarenal hemodynamics is based on two different techniques: the inert gas washout method and the analysis of the intracortical distribution of radioactive microspheres. Both methods gave nearly identical results: no change in the outer cortical flow fraction (CP-I of the washout curves and OC/C, $OC_1/C_1/OC_2/C_2$, respectively, in the microsphere experiments) as compared with control conditions, in hypoxic as well as in hypercapnic animals. The intrarenal distribution of blood flow (IDBF), therefore, stayed constant in spite of marked changes in renal vascular resistance. In view of the problems involved in the analysis of IDBF, the good agreement between both methods is reassuring. The inert gas washout method as a means of analyzing IDBF and local intrarenal blood flow rates has repeatedly been subject to criticism in the past [15, 16]. There is sufficient evidence, however, for the validity of the method in assessing mean renal blood flow ($\overline{F}$) and cortical blood flow [17–19]. Still, it was this criticism and the problem of correlating the components of the washout curve with specific areas of the kidney that led us to complement the inert gas washout data with additional information based on the intracortical distribution of radioactive microspheres. In applying this technique to the rat, several sources of error had to be overcome in addition to the basic problems discussed by previous authors [16, 17, 20, 21]. Standardized sectioning of the renal cortex and the effects of the microspheres on renal function and renal hemodynamics proved to cause the main difficulties. The separation of the outer and inner cortical layers followed a "midcortical" line judged by eye. In view of the steep increase in glomerular density from the corticomedullary border to the peak cortex (which is equal to the outer cortical layer of maximum microsphere density [16], Table 3), relatively small errors in judging this midcortical line can be expected to cause important artifactual changes in microsphere distribution. For this reason, the outer cortical fraction of MS (OC/C) was calculated and presented as the ratio of control injection/experimental injection (Table 4). Ideally, in reproducibility studies, this ratio should be close to unity. With the amounts of microspheres necessary to minimize counting errors and the problems of nonrandomness and poor mixing, however, a consistent though small drop in outer cortical microsphere fraction was observed after the first microsphere injection, resulting in a $Q_{control/experimental}$ of 1.07, against which the hypoxia results were compared.

Both problems—the effects of variations in sectioning of the cortex and the influence of microsphere injection on intrarenal hemodynamics—have not been given detailed discussion in the previous attempts of applying the microsphere distribution technique to the rat kidney [22–24], although the differing ratios of the numbers of microspheres trapped in the outer vs. the inner cortex (1.2 ± 0.1 reported by Källskog [24], 2.5 ± 0.3 observed by Hsu, [22]) as well as the reproducibility studies in a small number of animals ([22], $Q_{control/repeat} = 1.18$) seem to confirm the difficulties. Based on the additional microscopic analysis of microsphere distribution and with the restrictions and corrections indicated above, we feel that the microsphere distribution technique is an adequate method to study changes in intracortical distribution of blood flow in the rat kidney.

There is a considerable body of information pointing to the vasoconstrictor effects of hypoxia. The demonstration, therefore, of a clear-cut renal vasodilatation under conditions of acute and pure hypoxia (no change in P_{CO_2}) deserves some comments. Acute systemic hypoxia has been found to cause an increase in arterial pressure and vascular resistance in anesthetized dogs [25–27] and cats [28]. An increase in renal vascular resistance has been ascribed to an

increase in sympathetic discharge [26, 28] or adrenal medullary secretion of norepinephrine-like material [29]. In anesthetized animals, however, the pattern of vascular response to acute hypoxia is by no means uniform [27]. In contrast to the above reports, a depression of vasomotor responses to norepinephrine and angiotensin and local autoregulatory vasodilatation as a consequence of oxygen lack have been shown to oppose the sympathoadrenal stimuli, resulting, under appropriate conditions, in a reduced resistance to blood flow through most systemic vascular beds [30–35]. Thus, our findings of a decrease in renal vascular resistance with little or no change in total renal blood flow, despite a significant drop in systemic arterial pressure, are in good agreement with previous observations [36]. That this pattern is not simply the expected response of the renal vasculature to the reduction in systemic blood pressure is evident from our own observations of a marked *increase* in renal vascular resistance in rats and dogs with hemorrhagic hypotension [37].

In the present series of experiments, hypoxic vasodilatation occurred without appreciable changes in intrarenal or intracortical distribution of blood flow. This pattern of intrarenal hemodynamic changes differs from that observed in the anesthetized dog under conditions of pharmacologically induced renal vasodilatation: bradykinin and acetylcholine have been shown to cause a reduction in outer cortical and an increase in inner cortical flow fraction [38, 39].

In relating the findings of this study to the clinical problem of kidney function in acute respiratory failure and in comparing the present observations with other reports, a number of important points should be considered. First, the induction of hypoxia and hypercapnia, as well as the techniques used to analyze renal hemodynamics required experiments in the *anesthetized animal*. There is ample evidence that general anesthesia influences cardiovascular functions and, more specifically, renal hemodynamics [40–43]. Thus, the interference of barbiturates with the response to endogenous vasopressors (norepinephrine, angiotensin II) added to the hypoxic inhibition of vasoconstrictor responses [30–32] may account for the marked hypotensive effect of relatively minor degrees of hypoxia. For similar reasons, barbiturate anesthesia may have accentuated the hemodynamic changes in hypoxia and may have blunted the effects of hypercapnia. Furthermore, in the present experiments, access to the thoracic aorta and left ventricle was gained through one of the carotid arteries. Although the contralateral vessel was not touched, the resulting stimulation of the carotid baroreceptors may account for the increase in MAP and renal vascular resistance and the decrease in mean renal blood flow observed in our control groups as compared with our previous observations [14, 44]. Reactions to acute hypoxia, such as a decreased vasoconstrictor response to norepinephrine or angiotensin II, would therefore start at an increased level of renal nerve activity and sympathetic renal vasoconstriction [45]. This mechanism, again, may have accentuated the effects of hypoxia while blunting those of hypercapnia.

The finding of an increase in renal vascular resistance under conditions of a P_{CO_2} of 106 mm Hg (series E) agrees with previous observations in dogs and cats [28, 46]. This effect, which seems to occur with severe hypercapnia only, has been attributed to sympathoadrenergic stimuli [46, 47]. Our experiments were not designed to investigate the mechanisms causing renal vasoconstriction or vasodilatation. It should be noted, however, that severe hypercapnia in the present experiments did not alter the intrarenal or intracortical distribution of blood flow. In contrast, previous studies from this and other laboratories using identical techniques (inert gas washout) revealed a significant decrease in CP-I flow fraction, brought about by sympathoadrenergic stimuli [14].

On the basis of these studies, we conclude that moderate hypoxia in the anesthetized rat causes significant renal vasodilatation not associated with significant changes in intrarenal distribution of blood flow or renal excretory function. Similarly, renal vasoconstriction due to acute hypercapnia does not alter IDBF. Oliguria and anuria, observed under conditions of severe hypoxia with marked arterial hypotension, is not due to, nor accompanied by, renal vasoconstriction but occurs in spite of a significant decrease in renal vascular resistance. Acute renal failure, in these animals, must therefore be due to a systemic blood pressure that is insufficient to maintain glomerular filtration.

Acknowledgments

This study was supported by grant number 3.495.0.75 of the Swiss National Science Foundation. We acknowledge the help of Dr. Bruno Egloff who provided the stained histological sections, Prof. H. Bachofen who gave valuable help and criticism of the manuscript, Miss Françoise Simmler who gave advice and support with the counting techniques, and Drs. G. Ayer and H. P. Rentsch who were of great help in the initial stages of these studies.

Reprint requests to Dr. Bruno Truniger, Department of Medicine, Kantonsspital, CH-6000 Luzern, Switzerland.

References

1. GRANBERG PO: Effect of acute hypoxia on renal hemodynamics and water diuresis in man. *Scand J Clin Lab Invest* 14 (suppl. 63): 1–62, 1962
2. SMITH HW: *The Kidney: Structure and Function in Health and Disease*. New York, Oxford Univ Press, 1951, p. 441
3. THURAU K: Renal Na-reabsorption and O_2-uptake in dogs during hypoxia and hydrochlorothiazide infusion. *Proc Soc Exp Biol Med* 106:714–717, 1961.
4. GUIGNARD JP, TORRADO A, GAUTIER EG: Renal function in respiratory distress syndrome. *J Pediatr* 88:845–850, 1976
5. BRUNS F, TODROFF R, LOSOS K: Mechanism of decreased renal blood flow and glomerular filtration during hypoxia. *VIth Int Congr Nephrol*, Florence, 1975, abstract No. 170
6. WARREN DJ, LEDINGHAM JGG: Measurement of intrarenal blood-flow distribution in the rabbit using radioactive microspheres. *Clin Sci Mol Med* 48:51–60, 1975
7. FORLAND M, PULLMAN TN, LAVENDER AR, IMPI A: The renal excretion of ethylenediaminetetraacetate in the dog. *J Pharmacol Exp Ther* 153:142–147, 1966
8. THORBURN GD, KOPALD HH, HERD JA, HOLLENBERG M, O'MORCHOE CCC, BARGER AC: Intrarenal distribution of nutrient blood flow determined with krypton-85 in the unanesthetized dog. *Circ Res* 13:290–307, 1963
9. GRANDCHAMP A, AYER G, SCHERRER JR, TRUNIGER B: Intrarenal hemodynamics of the rat kidney determined by the Xenon washout technique. *Nephron* 8:33–45, 1971
10. AYER G, GRANDCHAMP A, WYLER T, TRUNIGER B: Intrarenal hemodynamics in glycerol-induced myohemoglobinuric acute renal failure in the rat. *Circ Res* 29:128–135, 1971
11. JONES LG, HERD JA: Autoradiographic visualization of ^{85}Kr in the normal dog kidney. *Am J Physiol* 226:886–892, 1974
12. PEPELKO WE, DIXON GA: Arterial blood gases in conscious rats exposed to hypoxia, hypercapnia, or both. *J Appl Physiol* 38:581–587, 1975
13. ALTLAND PD, BRUBACH HF, PARKER MG, HIGHMAN B: Blood gases and acid-base values of unanesthetized rats exposed to hypoxia. *Am J Physiol* 212:142–148, 1967
14. RENTSCH HP, AYER G, VALLOTTON M, ZIEGLER W, TRUNIGER B: Effects of angiotensin II and norepinephrine on intrarenal hemodynamics in the rat. *Eur J Clin Invest* 6:457–464, 1976
15. STEIN JH, BOONJARERN S, MAUK RC, FERRIS TF: Mechanism of the redistribution of renal cortical blood flow during hemorrhagic hypotension in the dog. *J Clin Invest* 52:39–47, 1973
16. KATZ MA, BLANTZ RC, RECTOR FC, SELDIN DW: Measurement of intrarenal blood flow: I. Analysis of microsphere method. *Am J Physiol* 220:1903–1913, 1971
17. SLOTKOFF LM, LOGAN A, JOSE P, D'AVELLA J, EISNER GM: Microsphere measurement of intrarenal circulation of the dog. *Circ Res* 28:158–166, 1971
18. LADEFOGED J, PEDERSEN F, DOUTHEIL U, DEETJEN P, SELKURT EE: Renal blood flow measured with Xenon-133 washout technique and with electromagnetic flowmeter. *Pfluegers Arch* 284:195–200, 1965
19. COSGROVE MD, EVANS K: The use of inert radioactive gases to measure blood flow in the perfused kidney, in *Blood Flow Through Organs and Tissues*, edited by BAIN WH, HARPER AM, London, E & S Livingstone, 1968, p. 429
20. BUCKBERG GD, LUCK JC, PAYNE DB, HOFFMAN JIE, ARCHIE JP, FIXLER DE: Some sources of error in measuring regional blood flow with radioactive microspheres. *J Appl Physiol* 31:598–604, 1971
21. WAGNER HN, RHODES BA, SASAKI Y, RYAN JP: Studies of the circulation with radioactive microspheres. *Invest Radiol* 4: 374–386, 1969
22. HSU ChH, KURTZ TW, PREUSS HG, WELLER JM: Measurement of renal blood flow in the rat. *Proc Soc Exp Biol Med* 149:470–472, 1975
23. LAMEIRE N, RINGOIR S: Influence de différents apports sodés sur la distribution intracorticale du flux sanguin rénal: Etude avec microsphères chez le rat. *J Urol Nephrol* 81:703–706, 1975
24. KÄLLSKOG O, ULFENDAHL HR, WOLGAST M: Single glomerular blood flow as measured with carbonized 141-Ce labelled microspheres. *Acta Physiol Scand* 85:408–413, 1976
25. DAUGHERTY RM, SCOTT JB, HADDY FJ: Effects of generalized hypoxemia and hypercapnia on forelimb vascular resistance. *Am J Physiol* 213:1111–1114, 1967
26. COSTIN JC, SKINNER NS: Effects of systemic hypoxemia on vascular resistance in dog skeletal muscle. *Am J Physiol* 218:886–893, 1970
27. KORNER PI: Circulatory adaptations in hypoxia. *Physiol Rev* 39:687–730, 1959
28. WEISSMAN ML, RUBINSTEIN EH, SONNENSCHEIN RR: Vascular responses to short-term systemic hypoxia, hypercapnia, and asphyxia in the cat. *Am J Physiol* 230:595–601, 1976
29. FOWLER NO, SHABETAI R, HOLMES JC: Adrenal medullary secretion during hypoxia, bleeding, and rapid intravenous infusion. *Circ Res* 9:427–435, 1961
30. DETAR R, BOHR DF: Oxygen and vascular smooth muscle contraction. *Am J Physiol* 214:241–244, 1968
31. HEISTAD DD, WHEELER RC: Effect of acute hypoxia on vascular responsiveness in man: I. Responsiveness to lower body negative pressure and ice on the forehead; II. Response to norepinephrine and angiotensin; III. Effect of hypoxia and hypocapnia. *J Clin Invest* 49:1252–1265, 1970
32. HEISTAD DD, ABBOUD FM, MARK AL, SCHMID PG: Impaired reflex vasoconstriction in chronically hypoxemic patients. *J Clin Invest* 51:331–337, 1972
33. ROSS JM, FAIRCHILD HM, WELDY J, GUYTON AC: Autoregulation of blood flow by oxygen lack. *Am J Physiol* 202:21–24, 1962
34. HADDY FJ, SCOTT JB: Metabolically linked vasoactive chemicals in local regulation of blood flow. *Physiol Rev* 48:688–707, 1968
35. BACHOFEN M, GAGE A, BACHOFEN H: Vascular response to changes in blood oxygen tension under various blood flow rates. *Am J Physiol* 220:1786–1792, 1971
36. DAUGHERTY RM, SCOTT JB, DABNEY JM, HADDY FJ: Local effects of O_2 and CO_2 on limb, renal, and coronary vascular resistances. *Am J Physiol* 213:1102–1110, 1967
37. SANDOZ P, GRANDCHAMP A, TRUNIGER B: Intrarenal hemodynamics in rats during hemorrhagic hypotension. *Eur J Clin Invest* 4:229–234, 1974
38. STEIN JH, FERRIS TF, HUPRICH JE, SMITH TC, OSGOOD RW: Effect of renal vasodilatation on the distribution of cortical blood flow in the kidney of the dog. *J Clin Invest* 50:1429–1438, 1971
39. MCNAY JL, ABE Y: Redistribution of cortical blood flow during renal vasodilatation in dogs. *Circ Res* 27:1023–1032, 1970

40. WARREN DJ, LEDINGHAM JGG: Renal circulatory responses to general anaesthesia in the rabbit: Studies using radioactive microspheres. *Clin Sci Mol Med* 48:61–66, 1975
41. COX RH: Influence of pentobarbital anaesthesia on cardiovascular function in trained dogs. *Am J Physiol* 223:651–659, 1972
42. BURGER BM, HOPKINS T, TULOCH A, HOLLENBERG NK: The role of angiotensin in the canine renal vascular response to barbiturate anaesthesia. *Circ Res* 38:196–202, 1976
43. CONGER JD, BURKE TJ: Effects of anaesthetic agents on autoregulation of renal hemodynamics in the rat and dog. *Am J Physiol* 230:652–657, 1976
44. KENDRICK E, OBERG B, WENNERGREN G: Vasoconstrictor fibre discharge to skeletal muscle, kidney, intestine and skin at varying levels of arterial baroreceptor activity in the cat. *Acta Physiol Scand* 85:464–476, 1972
45. IRIUCHIJIMA J, WILSON MF: Sympathetic vasoconstrictor activity to the kidney in carotid occlusion pressor reflex. *Proc Soc Exp Biol Med* 131:189–192, 1969
46. NORMAN JN, MACINTYRE J, SHEARER JR, CRAIGEN IM, SMITH G: Effect of carbon dioxide on renal blood flow. *Am J Physiol* 219:672–676, 1970
47. BERSENTES TJ, SIMMONS DH: Effects of acute acidosis on renal hemodynamics. *Am J Physiol* 212:633–640, 1967