Effect of Acute Local Renal Hypoxia on Renin Activity in Renal Venous Plasma[1] (35605)

J. A. SPATH, JR.,[2] R. M. DAUGHERTY, JR.,[3] J. B. SCOTT,[4] AND F. J. HADDY[4]

Departments of Physiology and Medicine, University of Oklahoma Medical Center, Oklahoma City, Oklahoma 73104

Huidobro and Braun-Menendez (1) studied renin secretion in dogs following either intravenous injection of KCN or ventilation with gas mixtures low in oxygen content. No renin was detected in the femoral arterial blood of dogs treated with KCN, nor was there a consistent change in renin release associated with hypoxia independent of a drop in systemic blood pressure. More recently, Skinner *et al.* (2) reduced the arterial oxygen saturation of dogs to 50–70%. While the systemic pressure of the dogs was elevated 10–20 mm Hg during the hypoxic period, no significant change in renal blood flow occurred, nor was there any increase in renin release during the hypoxic period. However, Oliver and Brody (3) demonstrated an increase in the granularity of juxtaglomerular cells of rats kept for 2 weeks in an atmosphere in which the oxygen content was gradually reduced to 7.8%. Since increased granularity of the juxtaglomerular cells has been suggested to be associated with increased renin secretion (4), a decrease in the P_{O_2} of the blood may provide an adequate stimulus for renin release. Indeed, Robertson *et al.* (5) demonstrated an increase in the size and number of granules of cultured human juxtaglomerular cells when the oxygen in the gas phase was reduced from 10 to 1%. However, the authors noted that the granules produced during hypoxia did not stain with antihuman renin antibody. The findings of Robertson *et al.*, therefore, suggest that hypoxia does not stimulate renin production. In this regard, the recent study of Braverman and Rostorfer (6) indicates that the lack of oxygen inhibits renin secretion. In their study, the secretion of renin was investigated using rat kidney slices. Significantly less renin was released into the medium when the slices were incubated in the presence of 100% N_2 than when they were incubated in the presence of oxygen. These authors suggested that *in vitro* renin release is an active process which, released from physiological control, proceeds maximally.

A literature search failed to reveal studies in which the *in situ* kidney was subjected to local hypoxia. Such a study was therefore initiated. Blood flow and pH were held constant in an attempt to more specifically reveal the influence of hypoxia per se.

The effect of local alterations in oxygen tension upon renal vascular resistance has been reported elsewhere by Daugherty *et al.* (7).

Methods. An extracorporeal lung circuit was interposed between the femoral artery and the renal artery of the dog. Ventilation of the extracorporeal lung circuit with a gas mixture containing 20 or 0% oxygen allowed

[1] This investigation was supported, in part, by Public Health Service Grants HE10778 and HE10879 and American Heart Association Grant 65G7. A preliminary report of this work appeared in Physiologist **9**, 290 (1966).

[2] Present address: Department of Physiology, P.O. Box 608, Medical College of Virginia, Richmond, Virginia 23219. This work was presented as partial fulfillment of the requirements for the Doctor of Philosophy degree and was carried out with the aid of a predoctoral fellowship of the National Institute of General Medical Sciences (1-F1-GM-21,-549-01A1).

[3] Present address: Departments of Physiology and Medicine, Michigan State University, East Lansing, Michigan 48823.

[4] Present address: Department of Physiology, Michigan State University, East Lansing, Michigan 48823. Dr. Scott is the recipient of U.S. Public Health Service Career Development Award 1-K4-46,-398-01.

rapid, controlled alterations of only the oxygen tension of the blood entering the renal artery while the oxygen tension in the rest of the blood of the animal remained normal. Samples of renal venous blood were collected before and during local changes in renal oxygen tension and/or blood flow. The samples of renal venous blood were treated and assayed in rats for vasoconstrictor activity.

Ten dogs were used in these experiments. The animals were anesthetized with sodium pentobarbital (33 mg/kg) and placed on a positive pressure respirator. The left kidney was exposed through a flank incision and a small polyethylene catheter was placed in the left renal vein via the testicular or ovarian vein to facilitate sampling of blood. Each animal was heparinized and the lung from another animal was inserted in the perfusion line between the right femoral artery and the left renal artery. The extracorporeal lung circuit was that described in detail by Daugherty *et al.* (7). By altering the oxygen content of the gas mixture ventilating the donor lung of the extracorporeal lung circuit, it was possible to alter locally only the oxygen tension of the blood entering the experimental kidney. Further, the blood flow to the left kidney could be changed at will by simultaneously increasing or decreasing the output of the two constant flow pumps which moved the blood through the circuit connecting the left kidney and the extracorporeal lung. Renal artery oxygen tension, renal perfusion pressure and the vascular pressure changes in the donor lung were recorded continually throughout the experiment as described elsewhere (7).

The donor lung was ventilated with a control gas mixture of 20% O_2–5% CO_2–75% N_2. The rate and stroke volume of the ventilator and the flow rates of the pumps were adjusted to produce a normal oxygen tension, pH, and perfusion pressure in the blood entering the renal artery; and normal pulmonary artery and venous pressures in the donor lung. Ventilation of the donor lung with the control gas mixture continued for at least 8 min after the renal perfusion pressure and renal arterial oxygen tension reached a steady value. Renal arterial oxygen tension and/or renal blood flow were altered in four subsequent periods. Each experimental period was maintained for an average of 13 min during which time a new steady-state condition was achieved with respect to renal perfusion pressure and renal arterial oxygen tension. In period 2, flow was held constant at the control level, but the donor lung was then ventilated with 5% CO_2–95% N_2. In period 3, renal arterial blood flow was still held constant at the control level, and the donor lung was again ventilated with the control gas mixture. In six of the animals, renal blood flow was severely reduced in period 4 by decreasing the pump outputs. The donor lung, however, continued to be ventilated with the control gas mixture. In the last experimental period, period 5, flow was continued at the same low level as in period 4, but ventilation of the donor lung was once again carried out using 5% CO_2–95% N_2. During each experiment six samples of renal venous blood (5 ml each) were obtained for assay of renin activity. A sample was obtained just prior to cannulation of the left renal artery and the other samples were drawn just before the conclusion of each of the experimental periods.

In four of the animals, the manipulations of periods 4 and 5 were reversed, *i.e.*, the experimental kidney was subjected to a period of combined low flow and hypoxia before an experimental period in which ventilation of the donor lung was returned to the control gas mixture. Further, in three of these four experiments control conditions were reinstituted rapidly after the last experimental period and an additional blood sample obtained 30 sec later.

Treatment of samples and assay for renin activity. Each sample of renal venous blood was immediately placed into a polyethylene tube containing 0.5 ml of 18×10^{-3} *M* ethylenediaminetetraacetic acid (disodium salt). All samples were immediately placed in an ice bath. Each sample remained in the ice bath until the collection of all samples was completed. Following centrifugation at 4°, the supernatant plasma of each sample was incubated at 37° for 4 hr. After incubation, the plasma samples were rapidly frozen and kept frozen until assayed for renin activity in anesthetized, vagotomized rats af-

TABLE I. Average ± SE of Renal Perfusion Pressure, Renal Blood Flow, and Renal Arterial Oxygen Tension Before, During, and After 16.9 min of Renal Hypoxia and of the Increase in the Assay Rat Pressure Following Injection of 0.2 ml of Renal Venous Plasma Obtained at the Same Time ($n = 10$).

Control			Hypoxia			Postcontrol		
P[a] (mm Hg)	F[b] (ml/min)	P_{O_2}[c] (mm Hg)	P (mm Hg)	F (ml/min)	P_{O_2} (mm Hg)	P (mm Hg)	F (ml/min)	P_{O_2} (mm Hg)
				Kidney				
128	102	105	94	102	6	134	102	104
±10	±9	±5	±8	±9	±1	±8	±9	±4
				Rat				
15[d]			18			19		
±2			±2			±2		

[a] P = renal arterial perfusion pressure and maximum rise in rat diastolic pressure.

[b] F = renal blood flow.

[c] P_{O_2} = renal arterial oxygen tension.

[d] The response to the plasma obtained prior to onset of perfusion was 12 ± 3.

ter the method of Skinner *et al.* (2).

Results. Table I shows that, at constant blood flow, ventilation of the donor lung with 5% CO_2–95% N_2 for 16.9 min produced a fall in renal perfusion pressure. The renal hypoxia was severe; the oxygen tension of the perfusing blood fell to 6 mm Hg. Bioassay of the plasma sample drawn during this period revealed a renin activity which was not significantly different from that in the control samples (periods 1 and 3).

Table II presents data from the six animals subjected to low flow and low flow plus hypoxia in periods 4 and 5, respectively. As expected, reducing the flow for 16.3 min produced a fall in perfusion pressure and an increase in renin activity. However, superimposition of hypoxia for 14.5 min on the low flow failed to further change these variables.

Periods 4 and 5 were reversed in four animals in an effort to determine the effect of the experimental sequence on renin activity. Simultaneous reduction of flow to 24 ml/min and oxygen tension to 2 mm Hg for 14.2 min in period 4 produced essentially the same fall in perfusion pressure (132 to 27 mm Hg) and increase in renin activity (20 to 31 mm Hg), seen when only flow was reduced (Table II). Removal of hypoxia, flow remaining at 24 ml/min, failed to significantly affect renin activity (39 mm Hg), though arterial pressure rose slightly (27 to 41 mm Hg). The renin activity in the sample obtained 30 sec after the renal blood flow returned to the control level appeared to be no different than that found in periods 4 and 5.

Discussion. These data fail to provide evidence for an acute effect of oxygen on renin activity. Reduction of the oxygen tension did not significantly influence the renin activity of renal venous plasma either at normal flow or at low flow. Furthermore, the increase in renin activity produced by reduction in flow was not different in the presence and absence of oxygen.

These findings do not mean that the oxygen tension is without effect on renin secretion. It is possible that in these experiments compensatory factors obscured a direct effect. Thus, if renin secretion is an active aerobic process (6) which is modulated by factors such as pressure (2) and sodium concentration at the macula densa (8, 9), then it is possible to see how a direct depressant effect could be obscured in these experiments. As shown in Table I, perfusion pressure fell by 34 mm Hg during the hypoxic period. If it be assumed that there are baroreceptors for renin secretion and that the hypoxic dilation occurred downstream to these baroreceptors, there would then be a force present tending to obscure a direct depressant effect. Furthermore, if hypoxic dilation altered renal function such that decreased sodium was seen at the macula densa, another compensatory fac-

tor would be present.

The experiments at low flow make the possibility of such compensatory factors less likely but do not rule them out. With low flow, hypoxia did not change perfusion pressure and perfusion pressure was too low for urine formation (Table II). However, it is still possible that minor changes in pressure and sodium concentration occurred at the presumed respective receptor sites (pressure rose slightly when hypoxia was removed at low flow in the four animals).

Furthermore, it should be emphasized that hypoxia was limited to roughly 15 min in these experiments. It is possible that changes in renal venous plasma renin activity would have occurred had the preparation been such as to allow more prolonged application of hypoxia.

Finally the observation that increased renin activity appeared when renal perfusion pressure approximated 30 mm Hg in the presence or absence of oxygen suggests that, for short periods, oxygen delivery and filtrate formation are not obligatory for the continued appearance of renin activity in renal venous blood.

Summary. Samples of renal venous plasma were obtained from dogs in which a lung from another dog was interposed in the renal artery. The lung was ventilated with various gas mixtures. In this way it was possible to locally alter the oxygen tension and flow rate of the perfusing blood. Reduction of oxygen tension to 6 mm Hg for 17 min was without effect on renin activity. Reduction of blood flow raised renin activity and superimposition of near anoxia failed to alter this changed activity. Therefore, these studies do not provide evidence for an acute local effect of hypoxia on renin secretion.

TABLE II. Average ± SE of Renal Perfusion Pressure, Renal Blood Flow, and Renal Arterial Oxygen Tension Before, During, and After 17 min of Renal Hypoxia, at 16.3 min of Reduced Blood Flow, and at 14.5 min of Reduced Blood Flow Plus Hypoxia, and of the Increase in the Assay Rat Pressure Following Injection of 0.2 ml of Renal Venous Plasma Obtained at the Same Time ($n = 6$).

Control			Hypoxia			Postcontrol			Low flow			Low flow + hypoxia		
P[a] (mm Hg)	F[b] (ml/min)	P_{O_2}[c] (mm Hg)	P (mm Hg)	F (ml/min)	P_{O_2} (mm Hg)	P (mm Hg)	F (ml/min)	P_{O_2} (mm Hg)	P (mm Hg)	F (ml/min)	P_{O_2} (mm Hg)	P (mm Hg)	F (ml/min)	P_{O_2} (mm Hg)
Kidney														
135 ±12	111 ±11	102 ±8	95 ±13	111 ±11	7 ±1	136 ±9	111 ±11	101 ±7	29 ±4	22 ±4	91 ±6	28 ±3	22 ±4	2 ±2
Rat														
16[d] ±2			19 ±4			18 ±4			31 ±5			33 ±5		

[a] P = renal arterial perfusion pressure and maximum rise in rat diastolic pressure.
[b] F = renal blood flow.
[c] P_{O_2} = renal arterial oxygen tension.
[d] The response to the plasma obtained prior to the onset of perfusion was 14 ± 5.

1. Huidobro, F., and Braun-Menendez, E., Amer. J. Physiol. **137**, 47 (1942).

2. Skinner, S. L., McCubbin, J. W., and Page, I. H., Circ. Res. **15**, 64 (1964).

3. Oliver, W. J., and Brody, G. L., Circ. Res. **16**, 83 (1965).

4. Hartroft, P. M., Circ. Res. **12**, 525 (1963).

5. Robertson, A. L., Smeby, R. R., Bumpus, F. M., and Page, I. H., Circ. Res. **18** and **19**, Suppl. **1**, 142 (1966).

6. Braverman, B. H., and Rostorfer, H. H.,

Physiologist **13**, 154 (1970).

7. Daugherty, R. M., Jr., Scott, J. B., Dabney, J. M., and Haddy, F. J., Amer. J. Physiol. **213**, 1102 (1967).

8. Nash, F. D., Rostorfer, H. H., Bailie, M. D., Wathen, R. L., and Schneider, E. G., Circ. Res. **22**, 473 (1968).

9. Vander, A. J., Physiol. Rev. **47**, 359 (1967).

Received Jan. 14, 1971. P.S.E.B.M., 1971, Vol. 137.