PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 154, 60-64 (1977)

# Redistribution of Renal Cortical Blood Flow by Renal Nerve Stimulation and Norepinephrine Infusion[1] (39604)

R. W. GOTSHALL AND H. D. ITSKOVITZ

*Wright State University School of Medicine, Dayton, Ohio 45431; and The Medical College of Wisconsin, Milwaukee, Wisconsin 53226*

It has been suggested that redistribution of renal blood flow within the cortex of the kidney alters sodium excretion (1, 2). The mechanisms by which this intrarenal distribution of renal blood flow may be regulated have not been elucidated. The purpose of the present study was to investigate the effect of two renal vasoconstrictor stimuli, renal nerve stimulation (RNS) and norepinephrine (NE), on the intrarenal distribution of renal blood flow, and to locate the cortical sites involved in regulation of intrarenal blood flow distribution.

The isolated blood-perfused canine kidney was used so that perfusion pressure (PP) and renal blood flow (RBF) could be controlled. In addition, the use of the isolated organ system eliminated systemic reflexes which otherwise might have altered the effect of RNS or NE.

*Materials and methods.* The method for isolated perfusion has been described fully in previous reports (3, 4). Kidneys were obtained from pentobarbital-anesthetized (30 mg/kg) male mongrel dogs weighing 18–23 kg. Within 2-min of removal, the kidneys were perfused with 800–900 ml of autologous heparinized blood at a constant temperature of 37°. The systolic pressure was maintained at 130 mm Hg as control pressure by adjusting RBF with a pulsatile pump (Waters Co.). For those experiments involving renal nerve stimulation (RNS), the renal nerves were removed with the kidneys. To accomplish this, the renal nerves were isolated as they approach the aorticorenal ganglion and cut distal to the ganglion. After perfusion of the kidney was begun, the nerves were secured in bundle fashion and placed on stimulating electrodes connected to a square wave stimulator (Grass Instruments). Stimulation duration was 10 msec, frequency was 10 cps, and the voltage was adjusted to increase renal resistance as needed (usually 1 to 5 V).

Renal blood flow (RBF) was measured directly by timed collections of renal venous effluent. The intrarenal distribution of blood flow was assessed with radioactive-labeled microspheres (3M Company) according to the technique of McNay and Abe (5) as modified for the isolated organ system (6). The microspheres were injected into the perfusion circuit just proximal to the perfusion pump 8.4 m from the kidney. This ensured adequate mixing of the microspheres as demonstrated by the uniform distribution of radioactivity at each level of the cortex from outer to inner cortex. To determine the distribution of renal blood flow, the cortex was divided into four equal zones from outermost to innermost. Six tissue samples were taken from each of the four cortical zones, and analyzed for their isotopic activity. A maximum of four isotopes ($^{46}Sc$, $^{85}Sr$, $^{141}Ce$, $^{51}Cr$) was injected into any one kidney. Blood flows were calculated for the outer half of the renal cortex (outer two zones) and for the inner half of the renal cortex (inner two zones). This partition was used since the medullary vasculature in the canine kidney is derived from arterioles of the inner half but not of the outer half of the renal cortex, thus hemodynamic changes in the inner cortex reflect changes in the medullary circulation (7).

The experimental protocol was as follows. Distribution of intrarenal blood flow was measured during a control period with renal perfusion pressure at 130 mm Hg. Then, renal vasoconstriction was induced by RNS or NE (Levophed) infusion (250–500 ng/min into the renal artery). Three to five

[1] This study was supported by grants from the U.S. Public Health Service, Grant No. NHL 12677 from the National Heart and Lung Institute, and by the Wisconsin Heart Association.

minutes after vasoconstriction, intrarenal distribution of blood flow was again measured (this always occurred within 15 min of the control measurement). The intensity of the vasoconstriction was adjusted by altering the voltage of the stimulator or the dose of NE infusion. Two studies were done on 22 kidneys in each study. In the first, the vasoconstrictor stimulus was increased to change perfusion pressure from 130 mm Hg systolic to approximately 170 mm Hg systolic, then PP was adjusted back to 130 mm Hg systolic by reducing RBF with the pump. In the second study, the vasoconstrictor stimulus was again adjusted to increase PP to approximately 170 mm Hg systolic. Perfusion pressure was left at this level; thus, RBF remained constant.

Statistical analysis of the data was performed using Student's *t* test for paired observations (8).

*Results.* The functional characteristics of the isolated kidney during control periods are given in Table I. Renal blood flow was similar to that measured in anesthetized dogs, but glomerular filtration rate (clearance of creatinine) was about 30% less (9). The isolated kidney reabsorbed about 98% of the filtered sodium and 97% of the filtered water, slightly less than the kidney *in situ*. The isolated kidney concentrated the urine. These results are similar to previously published data from this laboratory utilizing the isolated kidney model (3).

The effects of RNS and NE on total RBF are shown in Table II. RNS and NE were adjusted in all cases to increase systolic pressure from 130 mm Hg to approximately 170 mm Hg. In those experiments where the pressure was subsequently returned to 130 mm Hg with the perfusion pump, RNS and NE significantly reduced RBF by 28 and 23%, respectively. In those experiments where the pressure was left at 170 mm Hg, RNS and NE had no significant effect on RBF. Thus, two experimental conditions (reduced RBF and constant RBF) were created during which the effects of RNS and NE on the intrarenal distribution RBF were measured.

TABLE I. FUNCTIONAL CHARACTERISTICS OF ISOLATED KIDNEYS DURING CONTROL PERIODS ($N$ = 15).

| RBF (ml/min) | GFR (ml/min) | Fractional excretion of Na (%) | Fractional excretion of $H_2O$ (%) | U/P osmolality |
|---|---|---|---|---|
| 150[a] ± 10.9 | 20.9 ± 1.0 | 2.6 ± 0.67 | 3.3 ± 0.59 | 1.57 ± 0.082 |

[a] Mean ± SE.

Figure 1 shows the data for RNS and its effect on outer and inner cortical flow and on the intracortical distribution of blood flow for both experimental conditions (reduced RBF and constant RBF). RNS which significantly reduced RBF (first panel of Fig. 1, solid lines) also significantly reduced absolute flow to the outer cortex (from 108 ± 8 to 82 ± 9 ml/min) and to the inner cortex (from 39 ± 6 to 24 ± 5 ml/min). Although flow was reduced in both areas, the reduction was greatest in the inner cortex as exemplified in the significant increase in fractional flow (percentage of total RBF) to the outer cortex (from 75 ± 2 to 79 ± 2%) and significant decrease in fractional flow to the inner cortex (from 25 ± 2 to 21 ± 2%) (second panel of Fig. 1, solid lines).

RNS which did not alter RBF (first panel

TABLE II. SYSTOLIC PRESSURE AND RENAL BLOOD FLOW DURING RENAL NERVE STIMULATION (RNS) AND NOREPINEPHRINE INFUSION (NE).

| | Systolic pressure (mm Hg) | | Renal blood flow (ml/min) | |
|---|---|---|---|---|
| | C | E | C | E |
| RNS | 130 | 130 | 147 ± 13[a] | 106 ± 14* |
| NE | 130 | 130 | 167 ± 13 | 128 ± 14* |
| RNS | 130 | 170 | 155 ± 15 | 156 ± 18 |
| NE | 130 | 170 | 194 ± 16 | 189 ± 18 |

[a] Mean ± SE. $N$ = 11 for each series of experiments.

* $P < 0.005$.

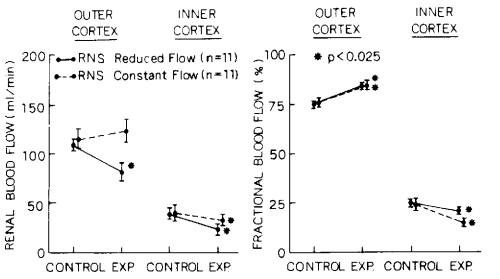


FIG. 1. Effect of renal nerve stimulation (RNS) on renal cortical blood flow distribution.

of Fig. 1, dashed lines) did not alter absolute flow to the outer cortex, although a tendency to increase was noted (from 115 ± 10 to 124 ± 14 ml/min). Inner cortical flow was significantly reduced (from 40 ± 7 to 32 ± 5 ml/min). This resulted in a significant increase in fractional flow to the outer cortex (from 76 ± 3 to 80 ± 2). Thus, when RNS decreased RBF, flow was reduced to both the outer and inner cortex with a redistribution of fractional blood flow from inner to outer cortex. When RBF was maintained constant in the face of RNS, only the inner cortex had a reduction in flow while the outer cortex had a tendency to increase flow, again with a redistribution of fractional blood flow from inner to outer cortex. These data demonstrate that RNS induced a greater vasoconstriction in the inner than outer cortical vasculature.

NE infusion produced the same pattern of results as RNS. This is shown in Fig. 2. NE infusion, which significantly reduced total RBF (first panel of Fig. 2, solid lines), reduced absolute flow to the outer cortex (from 116 ± 9 to 94 ± 11 ml/min) and to the inner cortex (from 51 ± 6 to 34 ± 5 ml/min). This resulted in an increase in fractional flow to the outer cortex (from 70 ± 2 to 74 ± 2%) and a decrease in fractional flow to the inner cortex (from 30 ± 2 to 26 ± 2%) (second panel of Fig. 2, solid lines). NE infusion which did not change RBF (first panel of Fig. 2, dashed lines) did not alter absolute flow to the outer cortex, although a tendency to increase was observed (from 133 ± 11 to 138 ± 13 ml/min). Inner cortical flow was decreased significantly from 61 ± 7 to 51 ± 7 ml/min. NE, with no change in RBF (second panel of Fig. 2, dashed lines), significantly increased fractional flow to the outer cortex (from 69 ± 2 to 73 ± 2%) and significantly decreased fractional flow to the inner cortex (from 31 ± 2 to 26 ± 2%).

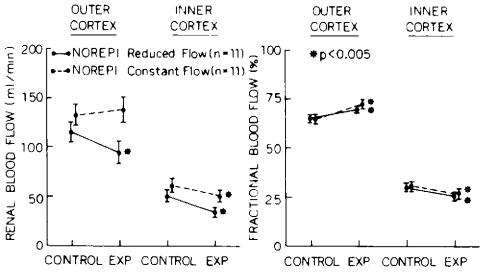


FIG. 2. Effect of norepinephrine infusion (NE) on renal cortical blood flow distribution.

NE thus produced similar effects as RNS, with a redistribution of fractional blood flow from the inner to the outer cortex in both conditions, when RBF was reduced and when RBF was not changed. It is apparent that NE, like RNS, has a greater vasoconstrictor effect on the inner cortical vasculature than on the cortical vasculature.

*Discussion.* In the present study, the use of the isolated perfused kidney permitted the evaluation of vasoconstriction on intrarenal hemodynamics under two experimental conditions. In the first series of experiments, vasoconstriction decreased RBF and perfusion pressure (PP) was held constant (130 mm Hg). These conditions reflect the *in situ* situation which may occur during the natural release of vasoactive hormones or increased sympathetic activity. Under these conditions, both RNS and NE caused a relative redistribution of RBF from the inner half of the cortex to the outer half of the cortex. Absolute flow to both areas was decreased, but there was a relatively greater decrease in the inner cortical flow. This suggests that the inner cortical vasculature responded to the stimuli with a greater vasoconstriction, although both RNS and NE caused vasoconstriction throughout the cortex.

In the second series of experiments, RNS and NE induced vasoconstriction elevated PP to approximately 170 mm Hg. Perfusion pressure was permitted to remain elevated as RBF was maintained constant. Under these conditions it is possible to define more clearly the sites of relative vasoconstriction. In a parallel circuit such as we have in the kidney, when RBF is constant any vasoconstriction will direct flow away from the area of greater resistance into an area of less resistance. Both RNS and NE directed flow from the inner half to the outer half of the cortex. In fact, under these conditions there was an increase in absolute flow to the outer cortex while decreasing flow to the inner cortex. This demonstrates that the vasoconstriction induced by RNS and NE is greatest

in the vasculature supplying the nephrons of the inner half of the cortex.

There have been reports that alpha-adrenergic stimulation may not produce homogeneous vasoconstriction throughout all of the nephrons. Pomeranz *et al.* (2) suggested that RNS, induced either by bilateral carotid ligation or splanchnic nerve stimulation, decreased outer cortical and increased inner cortical and outer medullary blood flow. Adrenergic stimulation also has been suggested to cause a redistribution of blood flow away from the outer renal cortex in humans with heart failure and in experimental models of sodium retention (1).

In contrast to these results, Stein *et al.* (10) and Rector *et al.* (11) have failed to demonstrate any effect of RNS (10) or NE (11) on the intrarenal distribution of blood flow. The disparate results obtained by the various investigators are most probably explained by the different technique used to measure the distribution of blood flow. In Barger's laboratory (1, 2) the gas-washout method was used, while in Stein's laboratory (10, 11), the radioactive microsphere method was used. As reviewed by Stein *et al.* (12) the gas-washout method measures total cortical flow instead of outer cortical flow, while the microsphere method distinguishes between the different areas within the cortex. Thus, a decrease in outer cortical flow as measured by gas-washout actually reflects a decrease in total cortical flow.

In our present study, both RNS and NE reduce total cortical flow in agreement with the gas-washout studies (1, 2). In addition, the microspheres in the present study have demonstrated that within the cortex itself, RNS and NE result in a relative redistribution of flow from the nephrons in the inner half of the cortex to those of the outer half of the cortex. Our study does differ from those of Stein and colleagues (10, 11) who also used the microsphere method, but showed no change in distribution of flow during RNS (10) or NE (11). The reason for the differing results is not clear. There are, however, two possibilities. One is the possible existence of a different response occurring in an isolated organ system as compared with that occurring *in situ*. If this is true, the reason for the difference needs to be investigated. The second reason may be the different level of stimulus used. In the present study, RNS and NE (250–500 ng/min) reduced RBF by 28 and 23%, respectively. While in the *in situ* experiments, RNS reduced RBF 40% (10) and NE (4 μg/min) reduced RBF 49% (11). These may possibly explain the differing results; however, further study is needed to clarify the differences.

It is evident from the data presented here that both RNS and NE can cause a redistribution of renal blood flow within the cortex and that this redistribution is from inner to outer cortical nephrons.

For a redistribution of blood flow to occur, there must be a differential response of the renal vasculature to vasoactive stimuli. Through the use of radioactive microspheres various investigations have been able to demonstrate a redistribution of renal blood flow between inner and outer cortical nephrons in response to a variety of vasoactive stimuli (3, 13–15).

Renal vasodilation with bradykinin (13) and prostaglandins (3) in the isolated perfused kidney, and acetycholine (14) and dopamine (15) *in situ* have been reported to redistribute fractional blood flow from outer to inner cortex. This redistribution is the opposite of that demonstrated in the present paper with vasoconstriction. These results indicate that there was a greater vasodilation of the inner cortical vasculature than the outer cortical vasculature, resulting in a redistribution of flow from outer to inner cortical nephrons.

The data from the present paper (vasoconstriction) and previous investigations (vasodilation) suggest that the inner cortical vasculature is more responsive to vasoactive stimuli than is the outer cortical vasculature. Thus, the vessels supplying the inner cortical nephrons are primarily responsible for the regulation of flow between inner and outer cortical nephrons. This differential response to vasoactive stimuli of the vasculature in the superficial and deep cortex represents an important mechanism by which the intrarenal distribution of blood flow can be regulated.

*Summary.* The effects of renal nerve stimulation (RNS) and norepinephrine infusion

(NE) on the intrarenal distribution of renal blood flow were studied in the isolated blood-perfused kidney. Both RNS and NE caused a redistribution of fractional blood flow from the inner half to the outer half of the renal cortex. Both vasoconstrictor stimuli resulted in a relatively greater vasoconstriction of the inner cortical vasculature. It is concluded that the inner cortical vasculature is more responsive to vasoactive stimuli and is the important locus for regulation of the distribution of renal cortical blood flow.

The authors wish to thank the staff of Allen Bradley Medical Science Laboratory for their help in completing this project.

1. Barger, A. C., Ann. N.Y. Acad. Sci. **139,** 276 (1966).
2. Pomeranz, B. H., Birtch, A. G., and Barger, A. C., Amer. J. Physiol. **215,** 1067 (1968).
3. Itskovitz, H. D., Terragno, A., and McGiff, J., Circ. Res. **34,** 770 (1974).
4. Berkowitz, H., Miller, L., and Itskovitz, H. D., Amer. J. Physiol. **213,** 928 (1967).
5. McNay, J., and Abe, Y., Circ. Res. **27,** 571 (1970).
6. Itskovitz, H. D., Hebert, L., and McGiff, J., Circ. Res. **32,** 550 (1973).
7. Fourman, J., and Moffat, D., "Blood Vessels of the Kidney," p. 58. Oxford and Edinburgh, Blackwell Scientific Publications (1971).
8. Steele, R., and Torre, J., *"Principles and Procedures of Statistics."* New York, McGraw-Hill (1960).
9. Selkurt, E., *In* "Handbook of Physiology" (W. Hamilton and P. Dow, ed.), p. 1483. Amer. Physiol. Soc., Washington, D.C. (1963).
10. Stein, J., Boonjaren, S., Mauk, R., and Ferris, T., J. Clin. Invest. **52,** 39 (1973).
11. Rector, J., Stein, J., Bay, W., Osgood, R., and Ferris, T., Amer. J. Physiol. **222,** 1125 (1972).
12. Stein, J., Boonjaren, S., Wilson, C., and Ferris, T., Circ. Res. **32** and **33** (Suppl. I), 61 (1973).
13. Terragno, N., McGiff, J., and Itskovitz, H. D., Fed. Proc. **32,** 353 (1973).
14. Stein, J., Ferris, T., Huprich, J., Smith, T., and Osgood, R., J. Clin. Invest. **50,** 1429 (1971).
15. Hardaker, W., Jr., and Wechsler, A., Circ. Res. **33,** 437 (1974).

Received June 22, 1976. P.S.E.B.M. 1977, Vol. 154.