THE EFFECTS OF SODIUM ORTHOVANADATE, NORADRENALINE AND ANGIOTENSIN II ON ISOLATED PERFUSED RAT KIDNEY GLOMERULAR-TUFT DIAMETER

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Summary : The effects of sodium orthovanadate, angiotensin II (A II) and noradrenaline (NA) were studied on the isolated perfused rat kidney (IPRK) and on the diameter of the glomerular capsule and tuft. Vanadate (4.5 μM), A II (20 nM) and NA (17.3 μM) increased the total resistance of the IPRK. There was a simultaneous increase in glomerular filtration rate with vanadate and A II, but a decrease with NA. The glomerular tuft/capsule diameter ratio decreased significantly from 0.85 (control) to 0.81, 0.81 and 0.78 for vanadate, A II, and NE treated kidneys, respectively. The decrease in ratio was associated with an increase in diameter of the glomerular capsule for A II and NE. This finding accompanied with simultaneous rise in TPR and GFR in the case of vanadate and A II, indicates that the post capillary efferent arteriolar vasoconstriction is a component in the mechanism of action with A II and vanadate. Evidence for such a component is less clear for NA because GFR decreases and TPR increases. Vanadate may affect both with a predominance on the efferent arteriole. The data indicate that histological measurements of glomerular size lead to a better understanding of the mechanisms of vasoactive drugs acting on the kidney.

Key words: orthovanadate noradrenaline angiotenisin II glomerulus kidney

INTRODUCTION

Sodium orthovanadate is a potent renal vasoconstrictor and diuretic agent; i.e., it caused a simultaneous rise in inulin clearance (GFR) and total peripheral resistance (TPR) in studies using the isolated perfused rat kidney (IPRK) (9). The combination of a rise in TPR and GFR suggests that vanadate exerts a preferential efferent arteriolar vasoconstrictor in the kidney. Such a mechanism has been proposed for angiotensin II (A II) (4). The present study of glomerular size in IPRK during vasoconstriction induced by sodium orthovanadate, noradrenaline (NA) and A II is an application of histochemical methods to differentially estimate afferent and efferent glomerular vasoconstriction.

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MATERIAL AND METHODS

Male Sprague–Dawley rats, (350–600 g) were used in the study. Methods of etting up the isolated perfused rat kidney (IPRK) preparation and measurement of various esponses (Table I) have been previously described (9).

The IPRK was allowed to equilibrate for a period of 30 min before the experimental udies were started. The control values obtained from the IPRK have been noted to be able for approximately 45 min following equilibration. Following a stabilized 10 min ontrol period, either 4.5 μM sodium orthovanadate, 20 nM A II, or 17.3 μM NA was dded to the perfusate. After 5 min, a 10 min collection and observation experiment as completed. Then the kidney was lifted from the organ chamber and immersed into quid nitrogen without interrupting the perfusion process. The frozen kidney was stored t –85°C. The control perfused kidneys were similarly collected without treatment with ne vanadate, A II, and NA. There was also a group consisting of non–perfused control idneys that were quickly frozen *in situ* as described previously (14).

Measurement of glomerular size : Sections (20 micrometer thickness) of frozen idney were made at –20°C using a microtome placed in a cryostat and were freeze–ried (14).

A dissecting microscope (Wild M–5) was used with a 10X eye piece adapted ith a 12 *mm* : 120 calibration scale (No. 255–501), and focused with a drum setting of 0X with a 2X additional objective giving a total magnification of 100X and a field of view f 2 *mm*. The scale on each large division was determined to be equal to 77 μ. Two istinct zones were noted in each glomerulus: a solid tuft and a surrounding clear zone ordered by the thin glomerular capsule (Fig. 1). The diameters measured included nat of the glomerular tuft and of the glomerular capsule. The majority of the glomeruli ere nearly round. Glomeruli showing markedly irregular shapes were not taken into onsideration during measurements. Glomeruli measured had a minimum diameter of 04 μ. The largest tuft diameter in a glomerulus was determined from a line extending om the base of the tuft through the center of the circle to the capsule, and the glomerular iameter was measured along this same line.

RESULTS

The functional viability of the IPRK was established in a series of experiments; ne control values are shown in Table I which also summarizes the effects of sodium

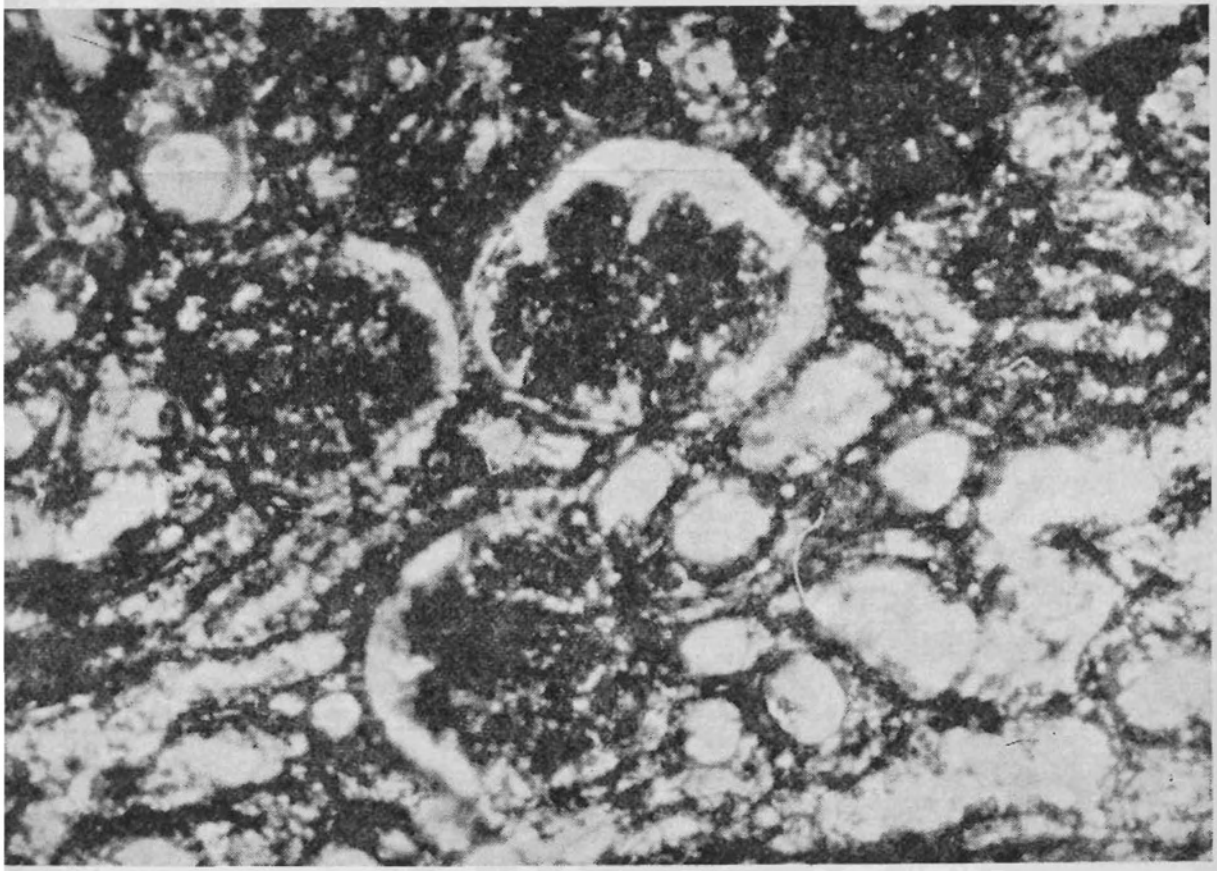

Fig. 1 : Freeze-dried rat kidney section showing kidney substructures.

orthovanadate, A II, and NA. With the vanadate and A II, there was an increase in GFR. However, NA decreased the GFR. Sodium reabsorption decreased in response to all three drugs. All the three drugs caused an increase in TPR and an enhanced urine flow. These data suggest that NE acts differently than vanadate or A II on the IPRK.

Glomerular capsule and tuft diameter measurements are summarized in Table II. The diameter of the glomerular capsule and the tuft was 149 and 126.6 μ, respectively, for the control perfused kidneys. The capsule diameter in nonperfused normal kidneys frozen *in situ* was intermediate among the IPRK samples. However, the tuft/capsule ratio was larger, 0.931, than IPRK.

TABLE I : Effect of Sodium Orthovanadate, Angiotensin II and Noradrenaline on the IPRK.

	Control	*Vanadate 4.5 μM*	*A II 20 nM*	*N.A. 17.3 μM*
	n = 17	*n = 4*	*n = 4*	*n = 4*
Urine flow, *μl/min*	42.1 (13.7)	146* (35)	445* (39)	250* (28)
G.F.R. (^{51}In), *μl/min*	381.1 (54.5)	755.3* (323.9)	1187.5* (264.9)	327.5* (44.2)
Na^+ excreted, *μEq/min*	5.4 (1.0)	16.7 (8.5)	44.4* (2.7)	26.2* (3.8)
K^+ excreted, *μEq/min*	2.0 (4.2)	3.8 (1.6)	8.4 (2.1)	1.9 (0.3)
Na^+ reaborption, %	95.6 (0.9)	87.0* (1.8)	71.0* (5.4)	48.4* (5.2)
TPR, KPa/l/min	538.5 (23.5)	731* (18)	952* (138)	844* (82)
KPa pressure	17.2 (0.1)	18.0 (0.4)	22.2* (1.4)	25.2* (0.8)

Values are means with S.E.M. in parentheses. n = number of perfusions.
*Value significantly different ($P < .05$) from control (t-test).

TABLE II : Glomerular size in isolated perfused rat kidney.

Treatment	*Number of kidney*	*Number of measurements*	*Glomerular capsule μm*	*Glomerular Tuft μm*	*Tuft/capsule X 100*
Perfused control	5	145	149.0 (1.6)	126.6 (1.2)	84.8 (0.6)
Vanadate	4	79	149.5 (1.8)	124.7 (1.6)	81.1 (1.0)*
Angiotensin II	4	170	164.7 (2.0)*	131.5 (1.3)*	80.5 (0.6)*
Noradrenaline	4	180	167.6 (1.5)*	131.7 (1.4)*	78.3 (0.7)*
Non-perfused control	3	90	154.3 (1.8)	143.7 (1.8)	93.1 (0.1)*

Values are means with S.E.M. in parenthesis. There was an average of 33 glomeruli measured per kidney.
*Value significantly different ($P < .05$) from control (t-test).

Under the influence of sodium orthovanadate, the capsular size was unchanged and the tuft size tended to decrease (Table II). The tuft/capsule ratio declined significantly from 0.848 to 0.811. A II caused an increase in capsular size to 164.7 μ and an increase in tuft size (Table II). The tuft/capsule ratio was significantly lower at 0.805. NA produced changes in glomerular size similar to that induced by A II (Table II). This occurred despite a dissimilar effect on GFR, Table I.

Glomeruli (6 per kidney) from the control IPRK kidneys (n=5) and those perfused with vanadate (n=4) were weighed on a quartz fiber balance by methods described previously (14). The weights were 28.3 $\pm$ 8.4 *mg* (mean $\pm$ SD) for kidneys perfused with the vanadate and 25.1 $\pm$ 8.7 *ng* for control perfused kidneys. In the group of nonperfused control kidneys, the glomeruli weights were higher at 59.0 $\pm$ 19.5 *mg*.

DISCUSSION

A number of pharmacological and physiological studies have utilized the IPRK. This laboratory has used it to study pharmacological effects of several drugs known to alter renal function. The effects of 4.8 μM vanadate on function of IPRK were similar to that previously reported (14). The effect of A II at 1.5–6 *ng/min* has also been noted before (4, 5). Both drugs increased TPR and GFR. However, Hofbauer *et al.* (6) noted that an infusion concentration of 0.2–0.7 *ng/ml/min* decreased GFR while increasing TPR. In contrast, in IPRK preparations made from spontaneously hypertensive rat, A II at 50 to 350 *ng/min* increased both GFR and TPR (13). NA decreased GFR while increasing TPR in the present study.

A II and NA have been studied in the *in situ* single nephron preparation. A II preferentially causes efferent arteriolar vasoconstriction (3, 7, 10) as does NA (10). However, both have a component of afferent vasoconstriction. In experiments where the pressure was allowed to rise, as opposed to experiments in which the aortic clamping prevented the drug–induced perfusion pressure change, NA had a relatively larger afferent vasoconstriction component than A II (10).

The diameter of the glomerular tuft should be increased upon infusion of A II and NA owing to efferent arteriolar vasoconstriction. In the present study the tuft was increased with A II and NE (Table II). Therefore, the histological measurements lend further support that A II and NE induce efferent arteriolar vasoconstriction. However, the vanadate did not increase tuft diameter, suggesting that any effect it may have on efferent arterioles may be different from A II and NE.

The glomerular capsule diameter was increased with A II and NE, but not by vanadate (Table II). The observed increase in TPR and the reported efferent vasoconstriction would also predict the increased diameter of the capsule with A II and NA, but one should expect increased GFR. GFR was only increased by A II and actually decreased with NA. The effect of vanadate on TPR would also suggest an increased capsule diameter, unless there was a relative preferential vasoconstriction of the afferent arteriole rather than efferent which might maintain a relatively constant pressure in Bowman's space.

The tuft/capsule ratio was lower than control after treatment with the vanadate, A II and NE. This would indicate that pressure in Bowman's space was increased by all the three drugs but that the relative contribution of afferent and efferent arteriolar vascular tone was unequal.

The glomerular size data noted in the present study is reasonably comparable to other reported measurements in the rat kidney, (2,8,11).

Interestingly, mesangial cell contractions induced by prostaglandins and several other drugs lead to reduced GFR (12). Like the mesangial cell contractions, mitochondrial swelling in nephron substructures may help to reduce GFR (1). Therefore, additional mechanisms also seem to serve as determinants of GFR, TPR, sodium reabsorption and urine flow in IPRK studies.

In conclusion, the histological measurements on glomeruli from the vanadate, NA, and A II IPRK experiments indicate that all three drugs have an effect on glomeruli. The histological measurements taken with the pharmacological evidence support that A II primarily causes an efferent arteriolar vasoconstriction. A combination of afferent and efferent arteriolar (and probably the mesangial effects) may explain the effect of NA. The pharmacological responses with the vanadate show the least correlation with diameter measurements in the glomeruli suggesting that its effect is primarily on the efferent arteriole and within the nephron tubules.

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