Effects of Angiotensin Infusion on the Isolated Rabbit Kidney (35781)

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(Introduced by R. Gaunt)
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The responses of the kidney to angiotensin depend on its dosage and on the state of sodium and water balance (1–4). To eliminate extrarenal influences on renal hemodynamics and tubular function, the isolated kidney, perfused at a constant pressure, was used to study the action of angiotensin on various parameters. In former experiments we showed that renal blood flow (RBF) of the isolated rabbit kidney, perfused with an angiotensinogen-free medium, was constant for the total period of 90 min, whereas glomerular filtration rate (GFR) decreased slightly (5). When small amounts of rabbit angiotensinogen were added to the perfusion fluid, GFR and filtration fraction (FF) increased (6). Since this effect was not observed with renin-depleted kidneys taken from rabbits pretreated with DOCA and salt, it was assumed that endogenous renin, by releasing angiotensin, acted on the efferent arterioles and in this way affected GFR. To support this interpretation, we studied the action of exogenous angiotensin on GFR and FF in the isolated rabbit kidney.

Materials and Methods. Rabbits of both sexes, weighing 2.5 to 3.5 kg, were anesthetized with pentobarbital (30–50 $mg \cdot kg^{-1}$ iv). Polyethylene catheters were placed into the ureter and into the artery of the left kidney. The left renal artery was connected to the perfusion system, and the blood was washed out with Tyrode's solution. Subsequently the kidney was transferred into a moist chamber. The perfusion fluid was free from angiotensinogen and consisted of Tyrode's solution containing 4 g of purified bovine albumin/100 ml (Behring-Werke, Marburg), to which washed rabbit erythrocytes had been added, resulting in a hematocrit of 40 vol%. The closed perfusion system had a total volume of 70 ml, and the perfusion was maintained by a peristaltic pump (Harvard Apparatus Mod. 1210). The perfusion fluid was oxygenized by a perspex disc oxygenator, with a gas mixture of 95% O_2 and 5% CO_2. All tubes were of silicon material. The whole perfusion system and the moist chamber were kept at a temperature of 38°. With the aid of a Statham transducer (Mod. P 23 Db), perfusion pressure was recorded at a tube connected to the cannula in the renal artery. During the total perfusion period, the pressure was kept constant by means of an electronic device regulating the perfusion volume. Variations in renal vascular resistance were compensated by changes in perfusion volume. Perfusion pressure was 97.1 ± 3.0 (SD) mm Hg.

The venous effluent was collected in a siliconized funnel, from where it was directly returned to the oxygenator. The same was done with the urine. The perfusion volume was measured directly by a glass cylinder every 2 min. The urine volume was measured by a glass cylinder during the clearance periods. Urine samples taken for analyses were replaced by corresponding volumes of Tyrode's solution.

GFR was determined by inulin clearance after inulin had been added to the perfusion fluid, resulting in a concentration of 0.5 $mg \cdot ml^{-2}$; the resorcinol method (7) was used for inulin determination. Sodium in urine was measured by flame photometry (Zeiss PMQ II).

After the kidney had been connected to the perfusion system, it was constantly perfused for 60 min. From the 60th to the 70th min, Val^5-angiotensin II-amide (Hyper-

tensin CIBA), in a dose of 0.1 ng·ml^{-1}·min^{-1} was infused into the tube connected to the renal artery. Samples of urine and of perfusion fluid were taken 2 min before starting the angiotensin infusion, at 2, 5, and 10 min after beginning the angiotensin infusion, and at 8 min after the infusion had been stopped (at the 78th min of the experiment). Eleven kidneys were studied in this way.

Results. Before the infusion of angiotensin was started, GFR was 3.37 ± (SD) ml·min^{-1} and renal "blood" flow 56.8 ± 7.8 (SD) ml·min^{-1}. Two min after beginning the infusion, GFR had increased by 14.5% and remained elevated at the 5th and 10th min of the infusion. After the infusion had been stopped, GFR fell to a level slightly below that before the beginning of the infusion (Table I). The FF showed corresponding variations; during the infusion period, it increased by 20.2, 19.2, and 26.3%, respectively, and 8 min after angiotensin had been stopped, it returned to a level slightly below the initial value. During angiotensin administration, perfusion volume was diminished by 4.2, 6.7, and 8.1%, respectively, and the renal vascular resistance (RVR) increased correspondingly. After the infusion, both parameters returned to their initial values. Urine volume increased continuously during the total perfusion period; the elevation was significant at 70 ($2p<0.01$) and 78 ($2p<0.05$) min of the experiment. Whereas urinary sodium excretion ($U_{Na}V$) was only slightly elevated during and after the infusion of angiotensin, absolute sodium reabsorption had increased by 21.3% ($2p<0.02$) within 2 min after the infusion had been started. During the infusion period, sodium reabsorption remained elevated, but it fell by 19.2% below the initial value after the administration of angiotensin had been stopped (Table I).

Discussion. In the isolated perfused rabbit kidney, Val^5-angiotensin II-amide infusion induced an increase in GFR and FF and simultaneously a rise in sodium reabsorption. A maximum effect was observed by 2 min after the beginning of the infusion and was maintained throughout the entire infusion period. Since the rate of angiotensin inactivation was not measured in our experiments, a

TABLE I. Effect of Val^5-Angiotensin II-Amide on Various Parameters of Isolated Perfused Rabbit Kidneys.[a]

After beginning of perfusion (min)	RBF (ml·min^{-1})	GFR (ml·min^{-1})	FF	Urine flow (ml·min^{-1})	U_{Na} (μEq·ml^{-1})	Na excreted (μEq·min^{-1})	Na reabsorbed (μEq·min^{-1})
58	56.8 ± 7.8	3.37 ± 0.74	0.099 ± 0.018	1.68 ± 0.51	131.4 ± 8.7	218.9 ± 71.0	283.3 ± 64.1
	Infusion of angiotensin II-amide started dose: 0.1 ng ml^{-1} min^{-1}						
60							
62	54.4 ± 7.1[b]	3.86 ± 1.05[b]	0.119 ± 0.030[b]	1.78 ± 0.62	129.5 ± 6.6	231.0 ± 86.0	343.6 ± 81.1[c]
65	53.0 ± 7.4[b]	3.75 ± 1.03	0.118 ± 0.030[c]	1.78 ± 0.51	128.2 ± 9.9	229.4 ± 70.8	330.0 ± 103.2
70	52.2 ± 7.5[b]	3.90 ± 1.13[c]	0.125 ± 0.037[b]	1.86 ± 0.58[b]	127.4 ± 9.0	239.4 ± 82.5	334.1 ± 103.9
	Infusion stopped						
78	56.8 ± 8.5	3.15 ± 0.97	0.095 ± 0.034	1.92 ± 0.54[d]	130.9 ± 6.8	241.9 ± 73.3	228.8 ± 85.8

[a] RBF, renal blood flow; GFR, glomerular filtration rate; FF, filtration fraction; and U_{Na}, urine sodium concentration. Values are means ± SD.
[b] *t* test, paired data: $2p < 0.01$; [c] $2p < 0.02$; [d] $2p < 0.05$.

cumulative effect cannot be excluded. However, as 8 min after cessation of the infusion all parameters had returned to, or even below, preinfusion levels, it is inferred that angiotensin at that moment was completely inactivated. It may be assumed, in addition, that an equilibrium between administration and inactivation of angiotensin was established, because the parameters that were followed did not vary significantly from min 2 to 10 of the infusion. The increase in renal vascular resistance was accompanied by an increase in GFR and FF, and therefore it is probable that angiotensin acted primarily on the efferent arterioles. The increase in tubular sodium reabsorption could be explained on the basis of hemodynamic effects, since a direct stimulating effect of angiotensin II on sodium transport in the proximal tubules seems unlikely (8, 9). Constriction of the efferent arterioles would elicit a rise in pressure proximal to and a reduction distal to the constriction, and consequently pressure in the peritubular capillaries would be expected to fall. By such a hemodynamic mechanism proximal tubular reabsorption of sodium may be enhanced without a direct effect of angiotensin on sodium transport in the proximal tubules.

Such a hypothesis is supported by the observation that in the rat, acetylcholine, after infusion into the aorta, elicits natriuresis (10). It was suggested that acetylcholine, by dilating the efferent arterioles and subsequently rising the hydrostatic pressure in the peritubular capillaries, may diminish proximal tubular reabsorption. In those experiments, an increase in pressure was measured in the proximal tubules, and consequently the effective filtration pressure was reduced. A reduction in tubular sodium reabsorption has also been observed after increasing the hydrostatic pressure in the peritubular capillaries by constriction of the renal vein (11).

Another factor, that could influence sodium reabsorption is the elevated FF observed in our experiments. An increase in FF may induce a rise in oncotic pressure in the peritubular capillaries, and simultaneously sodium reabsorption in the proximal tubules would be enhanced (12, 13).

The effects caused by angiotensin administered to the isolated perfused rabbit kidney correspond closely to those observed after the addition of angiotensinogen to the otherwise substrate-free perfusion fluid (6). Although it cannot be decided whether in the latter case renin reacted with the added substrate within the kidney or within the perfusion fluid, it may be concluded that, under the experimental conditions described, angiotensin released by endogenous renin from plasma substrate acts on the efferent arterioles similarly as infused angiotensin II-amide, thereby increasing GFR and FF. The enhanced sodium reabsorption found during angiotensin infusion may be explained by changes in peritubular hydrostatic and oncotic pressure and not necessarily by a direct effect on the tubular transport of sodium.

Summary. Isolated rabbit kidneys were perfused, at a constant pressure of 97.1 ± 3.0 mm Hg, with a suspension of 40 vol% rabbit erythrocytes in Tyrode's solution, to which 4 g% bovine albumin had been added. When, after a control period of 60 min, angiotensin II-amide, in a dose of 0.1 $ng \cdot m^{-1} \cdot min^{-1}$ was infused into the perfusion system for 10 min, GFR and FF increased during the infusion and fell to the preceding levels after it had been stopped. Urine volume and urinary sodium excretion were only slightly elevated. Absolute sodium reabsorption rose within 2 min after beginning the infusion, but fell below the initial value after angiotensin was turned off.

It is concluded that constriction of the efferent arterioles is responsible for the increase in GFR and FF, and that variations in sodium reabsorption may be the consequence of changes in renal hemodynamics rather than in tubular function.

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