Renal Angiotensinase Activity

ITS LOCALIZATION AND THE EFFECTS OF MERCURY

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ABSTRACT

A rat bioassay technique was employed to determine the localization and properties of the angiotensinase activity present in kidney homogenates of anesthetized rats. The angiotensinase activity per gram of renal cortex was four times greater than renal papilla or medulla. The complete destruction of proximal renal tubular cells by mercuric chloride resulted in losses of cortical angiotensinase activity averaging 40% of control values. The destruction of cells of both the proximal and distal renal tubules by sodium potassium tartrate caused a 90% reduction in angiotensinase activity. Marked reductions in cortical angiotensinase activity were measured in rats injected with meralluride 2 hours previously. The addition of meralluride to in vitro systems of normal rat kidney homogenates caused similar depressions of angiotensinase activity. The presence of reduced glutathione in the reaction media interfered with the ability of meralluride to inhibit angiotensinase and enhanced the activity of this enzyme to greater than normal levels in the absence of the mercurial. Ammonium ion in a concentration of .18 M inhibited angiotensinase activity of renal cortical homogenates by a moderate degree. When both meralluride and ammonium were present in the homogenate system their effects to depress angiotensinase appeared additive.

ADDITIONAL KEY WORDS

ammonium ion nephrotoxins
rat bioassay homogenates of cortex and medulla
meralluride glutathione

The renal pressor substance angiotensin is believed to be destroyed by an enzyme (or enzymes) angiotensinase. To date a specific angiotensinase has not been identified, although a number of proteolytic enzymes are known to possess angiotensinase activity. These include trypsin, chymotrypsin, aminopeptidase, and pepsin. The kidney is believed to be a rich source of angiotensinase activity although little is known concerning the nature of the enzymes involved, their locus, or their biochemical properties. In the present investigation data concerning these problems have been obtained by use of gross dissection, nephrotoxins, and enzyme inhibitors.

The results of these studies are indicative of a high concentration of angiotensinase activity in the renal proximal and distal tubules. In addition evidence was obtained consistent with the possibility that at least one renal enzyme with angiotensinase activity may be sulfhydryl-dependent.

Methods

Kidneys were removed from 250 to 300 g Sprague Dawley male rats following an intraperitoneal injection of pentobarbital (50 mg per kg). The kidneys were rinsed in cold saline and specimens of tissue obtained by gross dissection from papillary, medullary, and cortical areas. The specimens were weighed on a Mettler precision balance and homogenized in modified cold Tyrode's solution (0.4 ml per mg of renal tissue). The homogenates were centrifuged in the cold at 10,000 rpm for 20 min after which

The solution contained no glucose and was adjusted to pH 7.4 with HCl.
the supernatant containing the angiotensinase was separated and kept in ice until analysis.

NEPHROTOXINS
Mercuric chloride was administered intramuscularly to 3 male rats in a dosage of 0.005 mg per g of weight and to another 3 rats in a dosage of 0.1 mg per g of weight. The former dosage had been reported previously to damage the more distal areas of the proximal convoluted renal tubules and the latter to damage the entire proximal renal tubule of rats. An additional 2 rats received subcutaneous injections of sodium-potassium tartrate (2.5 g per kg) as a nephrotoxic agent after the rats were without food or water for a 48 hour period. The rats were killed 15 hours after the injection of the nephrotoxins and microscopic evaluation was made of more than 70 histologic sections prepared from three randomly selected areas of each damaged kidney. In conjunction with this, renal cortical tissue was obtained from each kidney and analyzed for angiotensinase activity.

MERALLURIDE IN VIVO
In 5 paired studies male Sprague Dawley rats (250 to 300 g) were injected intravenously with 0.05 ml of meralluride (®Mercuhydrin). Control rats received 0.05 ml of saline in place of the mercurial. Two hours later the rats were nephrectomized under pentobarbital anesthesia (50 mg per kg) and their renal cortical tissue analyzed for angiotensinase activity.

STUDIES IN VITRO
Meralluride in varying doses (Fig. 7) was added in vitro to 0.5 ml of incubates of renal cortical homogenates and 0.5 ml of a standard angiotensin solution (see below). The resultant effects on measurements of angiotensinase activity were compared with aliquot portions of the same homogenates and standard angiotensin solution without the mercurial. One half milligram of reduced glutathione dissolved in 0.067 M Tris buffer at pH 7.4 was added to each tube following the addition of 1.6 X 10^-8 to additional incubates from these rats with and without meralluride (Fig. 8) to determine the effects of sulfhydryl groups on angiotensinase activity. A study of the effects of ammonium on renal angiotensinase activity was considered since this ion is so closely associated with renal metabolic processes and since ammonium salts may alter the therapeutic effects of mercurial agents. Therefore 0.1 ml of 2.0 M ammonium ion at pH 7.4 (prepared by combining ammonium chloride with ammonium carbonate or dissolving ammonium chloride in 0.067 M Tris buffer) was added to other incubates to a final concentration of 0.18 M in the presence and absence of meralluride (Fig. 9).

ANGIOTENSINASE ASSAY
A standard solution of angiotensin* (2.5 μg per ml of 0.067 M phosphate buffer, pH 7.4) was freshly prepared at the time of each analysis. One half milliliter of standard angiotensin solution and an equal quantity of supernatant (prepared as above) were added to each of three test tubes and allowed to incubate at 37°C for 0, 15, and 30 min. The reactions were stopped by the addition of 0.4 ml of 0.1 N acetic acid and 2.5 ml of saline to each tube following which the tubes were placed in briskly boiling water for 10 min. The incubation mixtures were cooled and kept in ice until a bioassay could be performed for determination of pressor activity. The bioassay procedure utilized 400 to 450 g male Sprague Dawley rats which had undergone bilateral vagotomy and had received subcutaneous injections of atropine (0.08 mg) and pentolinium (5 mg in 2% PVP solution) to reduce sympathetic and parasympathetic influences. Following the injection of 0.1-ml samples from each tube into the femoral veins of such rats the resultant changes in blood pressure were recorded as registered on a mercury manometer attached to cannulas in the rats' carotid arteries. The angiotensinase activity of the homogenates was expressed in terms of the percentage by which control (0 incubation time) angiotensin pressor activity was reduced after 15 and 30 min of incubation.

Results
ANGIOTENSINASE ACTIVITY OF RENAL CORTEX VS. RENAL MEDULLA
Figure 1 illustrates the percentage reduction (angiotensinase activity) of control angiotensin pressor activity after incubating homogenates of rat renal cortex, medulla, and papilla with the standard angiotensin for 15 or 30 min periods. Little angiotensinase activity was demonstrated in the renal papilla. Following 15-min incubation with homogenates of papillary tissue of 4 rats, an average of 12% of the original pressor activity of angiotensin disappeared, and after 30 min the pressor activity had diminished by only 24%.

The angiotensinase activity of rat renal medulla was within a low range similar to the papilla. Studies of rat renal cortical angiotensinase activity were obtained in 11 rats.

*Hypertensin (Ciba) was kindly supplied by Dr. William E. Wagner, Ciba Pharmaceutical Products, Summit, New Jersey.
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Figure 1

Destruction of angiotensin by homogenates of rat kidney. The average reduction in pressor activity after incubating angiotensin with renal cortex for 15 or 30 min is approximately four times greater than that following incubation with medullary or papillary tissue. The number of observations is indicated in parentheses. The vertical line in each bar represents ±1 so from the mean.

After 15 min incubation, homogenates of cortical tissue destroyed an average of 49% of the angiotensin present in the incubation mixture, and after 30 min 79% of the angiotensin was destroyed. By using the present technique, the angiotensinase activity per gram of tissue was approximately three to four times greater in the cortex than in the papilla or medulla.

Effect of Nephrotoxins on Renal Angiotensinase Activity

Histologic section of rat renal cortex 15 hours after an intramuscular dose of mercuric chloride (0.1 mg per g of weight) is shown in Figure 2. Coagulation necrosis was observed in almost all cells of the proximal renal tubules with selective sparing of the distal tubular cells. Rats receiving a lower dose of mercuric chloride (0.005 mg per g) demonstrated similar lesions of entire proximal tubules but in general the damage was not as widespread throughout the kidney. The present experience differs from that previously reported in which lesions were limited to the distal portions of the proximal tubules following the low dose of mercuric chloride employed in these studies.

The loss of renal cortical angiotensinase activity associated with mercuric nephrotoxicity is illustrated in Figure 3. Angiotensinase activity in the 30-min incubates was decreased by an average of approximately 40% in 3 rats which received the higher dosage schedule of mercuric chloride. In the rats receiving the lower dose of mercuric chloride renal cortical angiotensinase activity was decreased to a lesser extent. Although there was some variation among the individually poisoned rats regarding the degree by which mercuric toxicity depressed angiotensinase activity, in general this correlated with the amount of tubular damage observed in histologic sections of the kidneys of these rats.

An example of the renal lesion observed in
Effect of nephrotoxins on the destruction of angiotensin by renal cortex. Note decreased angiotensinase activity of renal cortex following injury to proximal tubules by Hg Cl₂ and the marked reduction of angiotensinase activity following destruction of both proximal and distal tubules by Na-K tartrate. The number of observations is listed in parentheses. The vertical line in each bar is ±1 SD from the mean.

![Figure 3](http://circres.ahajournals.org/)

FIGURE 3

Extensive destruction of proximal and distal tubules following injection of Na-K tartrate. Glomerulus appears to be intact. (Hematoxylin and eosin, ×200.)

the 2 rats receiving sodium-potassium tartrate is shown in Figure 4. There was generalized destruction of both proximal and distal renal tubules. The glomeruli and vascular tissue appeared to remain relatively intact. As illustrated in Figure 3 angiotensinase activity disappeared by approximately 90% from the cortex of these rats.

**MERALLURIDE IN VIVO**

In each of 5 paired studies the injection of meralluride caused marked reductions in renal cortical angiotensinase activity of mercurial-treated rats in comparison to the saline-treated paired controls (Fig. 5). After 15 min incubation, the angiotensinase activity of mercurial-treated kidneys ranged from 33 to 65% of control values with an average of 50%; in the 30-min incubates the angiotensinase of mercurial-treated kidneys was 58 to 78% of control with a mean of 66%. Histologic sections of the kidneys of mercurial-treated rats revealed no obvious pathologic lesions by light microscopy when these depressions of enzymatic activity were measured (Fig. 6).

**MERALLURIDE IN VITRO**

By present methods 0.001 ml of meralluride (equivalent to 3.9 × 10⁻² mg of elemental mercury) caused marked reductions in renal angiotensinase activity when added in vitro to incubates of cortical homogenate and standard angiotensin solution (Fig. 7). Still greater degrees of inhibition were noted when the amount of mercurial was increased by a factor of 10 or 100, though the differences between the latter two did not appear significant. For reasons unknown at present, great variations in degree of angiotensinase inhibition were measured in the individual experiments where the lower concentrations of mercurial were employed. Nevertheless in each of these studies, meralluride caused obvious
Effect of intravenous mercurial (0.15 to 0.20 ml per kg) on angiotensinase activity of renal cortex. The mean angiotensinase activity of mercurial-treated rats was reduced markedly in comparison to paired controls. There were 5 observations. The vertical line in each bar represents ±1 so from the mean.

depressions in angiotensinase activity when compared with the control systems.

EFFECT OF REDUCED GLUTATHIONE

In 5 studies, 0.5 mg of reduced glutathione was added to the incubates in the presence of 0.01 ml of mercurial (3.9 × 10⁻¹ mg Hg). The addition of glutathione diminished the mercurial inhibition of angiotensinase activity in all 5 studies (Fig. 8) by an average of approximately 50 to 67%. In each case where reduced glutathione was added to incubates of renal cortex without the mercurial, striking increases in angiotensinase activity were measured, often to levels which had not been observed previously in these studies.

EFFECT OF AMMONIUM ION

The presence of 0.18 M ammonium ion in the reaction media reduced angiotensinase activity to an average of 60 to 70% of normal (Fig. 9). When both ammonium ion and mercurial (3.9 × 10⁻² mg Hg) were present, their effects to depress angiotensinase appeared additive. Under these circumstances the angiotensinase activity of homogenates was reduced to 20 to 25% of control values.

Discussion

The present demonstration in rats of little angiotensinase activity in the renal papilla or medulla and much greater activity in the cortex is consistent with a primary localization of angiotensinase to the cells of the renal convoluted tubules. The distribution of these cells within the kidney most accurately corresponds to the intrarenal distribution of angiotensinase. Little angiotensinase activity was measured in homogenates of rat renal papilla which to a large extent consists of the epithelial cells of collecting tubules. Similarly angiotensinase activity was low in medullary homogenates which in the rat largely consists of cells from the ascending and descending limbs of the loops of Henle. The greatest amount of angiotensinase activity was present in the renal cortex composed primarily of cells from the renal glomeruli and the proximal and distal convoluted tubules.
The loss of angiotensinase activity following the destruction of renal tubular cells by two different classes of nephrotoxins is additional evidence for the presence of angiotensinase in this area. There was a general correlation between extent of proximal tubular injury and loss of angiotensinase activity when differing doses of mercuric chloride were employed with a maximum reduction in angiotensinase of approximately 40% following the destruction of almost all proximal renal tubules by mercury. When both proximal and distal tubular cells were destroyed by sodium potassium tartrate the loss of cortical angiotensinase activity was increased to approximately 90%. The small percentage of angiotensinase activity remaining in this latter situation may have been the result of a failure to destroy all tubular cells by the tartrate or, more likely, could indicate the presence of a small amount of angiotensinase activity in nontubular cortical tissue.

The observation in present studies that meralluride may inhibit and reduced glutathione enhance the destruction of angiotensin raises the possibility that one or more enzymes possessing angiotensinase activity may be sulfhydryl-dependent. The mechanism by which ammonium ion may inhibit angiotensinase especially in combination with mercury is not known. It may be pertinent that ammonium ion has been demonstrated to stabilize proteins against proteolysis in concentrations similar to those employed in this study. Also ammonium ion has been reported to decrease the titer of measureable sulfhydryl groups on protein analysis7 and to enhance the binding of mercury to sulfhydryl groups by interfering with its reaction with other single ligand groups.8

One question raised by the present results...
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![Graph showing inhibition of angiotensinase activity by ammonium ion in the presence and absence of mercuric chloride.](image)

The inhibition of angiotensinase activity by ammonium ion in the presence and absence of mercuric chloride is illustrated. The number of observations is listed in parentheses. The vertical line in each bar represents ±1 so from the mean.

is whether or not the diuretic properties of the mercurial agents may be related in any way to an inhibition of renal angiotensinase activity. No data pertinent to this problem was obtained in the present investigation. However, since angiotensinase activity appears to be located in the renal tubule, and since the mercurials, ammonium salts, and angiotensinⅡ,10 each may affect renal tubular sodium and water metabolism, an investigation of the interrelationships among these substances may prove an interesting area of study in the future.

Acknowledgment

The authors are pleased to acknowledge the assistance of Dr. Arnold Rawson who reviewed the pathologic material and the helpful suggestions of Drs. James B. Wyngaarden, Calvin F. Kay, and Alfred M. Sellers in the preparation of this manuscript.

References

Renal Angiotensinase Activity: Its Localization and the Effects of Mercury
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Circ Res. 1966;19:507-513
doi: 10.1161/01.RES.19.3.507

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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World Wide Web at:
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