Angiotensin as a Possible Intrarenal Hormone in Isolated Dog Kidneys

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ABSTRACT
The distribution of renal blood flow was measured in isolated blood-perfused dog kidneys using radioactive labeled microspheres. The kidneys were perfused at a constant systolic pressure of 140 mm Hg. For the first 50 minutes of perfusion, flow to the outer zones of the renal cortex was 79 ± 2% (SE) of the total renal blood flow, and inner cortical blood flow accounted for 21 ± 2%. With continued perfusion, inner cortical blood flow increased progressively, reaching 34 ± 3% by 150 minutes—a time when renin substrate was depleted in the perfusates. Infusions of the tetradecapeptide renin substrate (5-50 μg/min) reestablished the fractional distribution of renal blood flow observed in the initial 50 minutes of perfusion, i.e., inner cortical blood flow was reduced significantly from 36 ± 2% to 23 ± 4% (P<0.05). In contrast, infusions of angiotensin II (1-10 μg/min) did not reduce the fractional distribution of renal blood flow to the inner cortex. These data provide evidence to support the hypothesis that angiotensin might be formed as an intrarenal hormone which participates in the regulation of deep cortical and medullary vascular resistances. Circulating angiotensin II does not appear to serve a similar function.

KEY WORDS
radioactive microsphere
angiotensin II
tetradecapeptide renin substrate
renal cortical blood flow
intrarenal distribution of blood flow
para-aminohippuric acid clearance

In previous studies of renal hemodynamics in isolated blood-perfused dog kidneys, we reported a gradual diminution in renal vascular resistance and an increase in renal blood flow during the initial 1-2 hours of perfusion (1, 2). The most marked increases in renal blood flow were inferred, from the analysis of ¹³¹I Xe washout curves and the observed diminished extraction ratios of para-aminohippuric acid (PAH), to be to the deep cortex and the renal medulla. Similar hemodynamic patterns in isolated perfused kidneys have been reported by other investigators (3). Since renin was released by the isolated kidney and renin substrate was used to the point of deficiency in the isolated perfusate at a time when these hemodynamic changes occurred, we suggested that these events might be related (1). Recent data of Krahé et al. (4) in isolated perfused rabbit kidneys are compatible with our results in the canine kidney. These investigators reported a similar release of renin and a similar dependence of renal vascular resistance on perfusate concentrations of renin substrate in their preparation. As a result of these experiments, we hypothesized that reduced plasma concentrations of renin substrate might decrease the efficiency of local generation of angiotensin at vascular sites within the kidney and thus give rise to alterations in renal hemodynamics. We also suggested that angiotensin might be formed as an intrarenal hormone which assists in the regulation of medullary vascular resistance. However, to a large extent our previous assessments of intrarenal hemodynamics depended on changes in clearances and extraction ratios of PAH. Since the transport of PAH might be dependent on factors other than renal blood flow, we needed to obtain additional measurements by other techniques to support our hypotheses. The recent introduction of radioactive microspheres for measurement of the intrarenal distribution of blood flow provided this opportunity. In the present paper, we refer to angiotensin as a potential intrarenal hormone, but we have not specified whether the hormone is angiotensin I or angiotensin II. There is some question whether angiotensin II is formed in the renal vasculature and whether angiotensin I is able to function as a vasoconstrictor hormone (5, 6). Until these problems are resolved, we felt that the more general term "angiotensin" was preferable.
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Methods

Dog kidneys were perfused with autologous blood according to methods published previously (1, 2) except that the blood was defibrinated rather than heparinized. The defibrinated blood was used to avoid a potential interference of heparin in the reaction of renin with its substrate (7). Systolic perfusion pressure was kept constant at 140 mm Hg, and mean renal perfusion pressure was maintained between 115 and 120 mm Hg. Total renal blood flow was measured directly by timed collections of the renal venous effluent. The intrarenal distribution of blood flow was assessed using microspheres 15–20 μ in diameter labeled except that the blood was defibrinated rather than heparinized. The defibrinated blood was used to avoid a potential interference of heparin in the reaction of renin with its substrate (7). Systolic perfusion pressure was kept constant at 140 mm Hg, and mean renal perfusion pressure was maintained between 115 and 120 mm Hg. Total renal blood flow was measured directly by timed collections of the renal venous effluent. The intrarenal distribution of blood flow was assessed using microspheres 15–20 μ in diameter labeled with 153Yb, 85Sr, 141Ce, or 51Cr (3M Company). The microspheres, 1–4 μ, were injected into the perfusion circuit just proximal to the perfusion pump 9.4 m from the kidney. Subsequent monitoring for radioactivity in the renal venous effluent was negative and consistent with complete entrapment of the microspheres in the kidney. At the end of each experiment, the kidneys were frozen and, within a day, 24 tissue specimens were obtained from four equal cortical zones (zone 1 was the outermost and zone 4 the innermost zone) according to techniques described by McNay and Abe (8). The isotopic content of each tissue specimen was determined using a Packard Autogamma counter, and the mean relative distributions of isotopes at various depths of the cortex were calculated (8). Injections of microspheres were made at various times during isolated perfusion in the control state and immediately before and 10–15 minutes after the beginning of infusion of synthetic tetradecapeptide renin substrate (Schwarz Mann) or angiotensin II (Hypertensin, Ciba). The synthetic preparation of renin substrate was used to ensure purity: it was uncontaminated by renin or other plasma proteins which might have been present if the renin substrate had been prepared by plasma extraction procedures. The infusions of renin substrate or angiotensin II commenced only after 100–150 minutes of isolated perfusion at a time when native renin substrate was severely diminished in the perfusate (1) and an increased percent of blood was flowing through deep cortical areas (Fig. 1). Either renin substrate or angiotensin II was added at rates which reduced renal blood flow by only 10–20 ml/min, i.e., 5–50 μg/min for renin substrate and 1–10 μg/min for angiotensin II. The infusion rates for renin substrate were within the range at which substrate is normally delivered to the kidney (approximate plasma concentration of renin substrate × approximate renal plasma flow = 0.27 μg/ml × 125 ml/min = 33.8 μg/min) (9). The high infusion rates for angiotensin II indicate some refraactoriness to its vasoconstrictor action in the isolated kidney.

Results

DISTRIBUTION OF FLOW IN ISOLATED KIDNEYS

The fractional distribution of blood flow at various times during isolated perfusion without hormonal additions is shown in Figure 1. Each bar signifies the mean of at least ten different perfusion studies for which measurements were obtained during that particular time interval. Outer cortical blood flow represents that fraction of the total renal blood flow to zones 1 and 2 and inner cortical flow is the fraction to zones 3 and 4. During the initial 50 minutes of isolated perfusion, mean flow to the outer zone accounted for 79 ± 2% of the total renal blood flow with only 21 ± 2% going to the inner zones. There was little change in the next 50 minutes, but beyond that time a highly significant shift of blood flow to deeper cortical areas occurred: the fractional renal blood flow to the inner cortex reached 34–37% after 100 minutes. This increase was not a consequence of increased outer cortical vascular resistance, but rather it resulted from a reduction of resistance in all cortical zones, especially in the deep cortex. Thus, in terms of changes in absolute blood flow, outer cortical blood flow increased from 90 ± 9 ml/min to 141 ± 11 ml/min in this period and inner cortical blood flow increased from 23 ± 2 ml/min to 73 ± 7 ml/min (P < 0.01).

Figure 2 illustrates changes in the distribution of blood flow in 11 experiments. Each straight line connects the percent of inner cortical flow measured at two separate time intervals in the same kidney. In this figure and the one which follows, only blood flow to the inner cortex is shown. The corresponding values for blood flow to the outer cortex can be calculated by subtraction from 100%. Note in every case that fractional blood flow to the inner cortex
Changes in the percent of inner cortical blood flow in 11 individual kidneys. Later measurement in each of 10 kidneys showed a higher percent of inner cortical blood flow. Note in 1 kidney the similarity of two measurements obtained within several minutes of each other.

was higher at the time of the second injection of microspheres.

INFUSIONS OF TETRADECAPETIDE RENIN SUBSTRATE

The distributions of flow in six isolated kidneys before and during infusions of tetradecapeptide renin substrate are shown in Figure 3. In five of six preparations, the percent of renal blood flow to the inner cortex decreased after substrate infusion \((P < 0.05)\). Thus, renin substrate reversed the usual pattern of an increase in inner cortical blood flow with time. The fractional blood flow to the inner cortex fell from \(30 \pm 2\%\) to \(23 \pm 4\%\) after substrate was added to the perfusate \((P < 0.05)\). The latter value \((23\%)\) was significantly less \((P < 0.05)\) than the fractional blood flow of \(34\%\) to the inner cortex measured at a similar time interval of perfusion \((100-200\) minutes\) without the addition of renin substrate (Fig. 1). Corresponding calculated values for absolute blood flow to the outer and inner cortical areas before and after the infusions of renin substrate revealed a reduction in blood flow which was confined to the inner cortex. Thus, outer cortical blood flow went from \(123 \pm 14\) ml/min to \(135 \pm 24\) ml/min after the substrate was added, whereas inner cortical blood flow diminished from \(68 \pm 11\) ml/min to \(44 \pm 10\) ml/min.

INFUSIONS OF ANGIOTENSIN II

The effect of angiotensin II on inner cortical blood flow in five isolated kidneys is also illustrated in Figure 3. In contrast to the effects of tetradecapeptide renin substrate on intrarenal distribution of blood flow, angiotensin II usually caused a further increase in fractional blood flow to the inner cortex. The mean inner cortical fraction after angiotensin II infusion was \(34 \pm 2\%\) in these five kidneys. The latter value is within the range for isolated kidneys at \(100-200\) minutes of perfusion without addition of angiotensin II. On the basis of these few experiments, we could not conclude whether angiotensin II caused any significant change in the distribution of blood flow in the isolated kidney, although our results suggested a vasoconstrictor effect which was greater in the outer cortex than it was in the inner cortex (Fig. 3). These impressions were also supported by the calculated changes in absolute flow to the outer and the inner renal cortex during infusions of angiotensin II. Outer cortical blood flow tended to decrease \((131 \pm 14\) ml/min to \(118 \pm 15\) ml/min\), but there was no similar effect in the inner cortex \((53 \pm 8\) ml/min to \(60 \pm 6\) ml/min\).

Discussion

In the present studies on the isolated perfused dog kidney, the tetradecapeptide renin substrate affected the distribution of intrarenal blood flow: inner cortical blood flow and, thereby, medullary...

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flow were reduced (the medullary vasculature including the vasa recta of dog kidneys is derived from the efferent arterioles of the inner cortical nephrons [10]). Although the tetradecapeptide renin substrate might have a slight vasoconstrictor effect of its own, its major hemodynamic effect results from its reaction with renin to form angiotensin (11). Since infusions of angiotensin II (extrarenal hormone) did not result in a similar renal hemodynamic pattern in the present or previous studies (12), we hypothesize that the response to the tetradecapeptide renin substrate resulted from an enhanced local production of intrarenal rather than extrarenal angiotensin. To explain a selective effect of renin substrate on inner cortical and medullary blood flow by enhancement of the intrarenal production of angiotensin, the enhancement would have to occur preferentially at medullary vascular sites. A preferential medullary site of formation of angiotensin is consistent with the following considerations of the enzymatic kinetics and physiology of the renin-angiotensin system and the anatomy of the renal circulation.

First, the enzymatic generation of angiotensin is influenced by the concentrations of renin and renin substrate, the pH, and the time of reaction (13). Each of these factors might be crucial to the quantity of angiotensin formed within the blood vessels of the kidney during the short renal transit time of blood. The pH of the medulla provides the optimum acidity, and its slow circulation time, perhaps the slowest of any region of the body (8-30 seconds compared with 2.3 seconds for the cortex) (14), provides a relatively long period of time which favors the medullary formation of high concentrations of angiotensin in response to the local secretion of renin. Also, the long medullary efferent arterioles and the vasa recta (401-4,700µm) (15), which are distal to juxtaglomerular sites of renin secretion, offer a lengthy and potentially responsive target for the vascular actions of that quantity of angiotensin which might be produced locally for this prolonged period. Comparative measurements of postglomerular efferent arterioles in the cortex reveal that these vessels are only 233 ± 85µm long (15). In view of the short cortical efferent arterioles and the rapid cortical circulation, there might not be enough time for sufficient angiotensin to be generated in cortical blood vessels to allow its action there as a local hormone. Since angiotensin has been reported to cause little vasoconstriction (16), it is not likely to affect the renal venous circulation, and the time available for it to form and act in the cortex might be considerably less than the 2.3-second cortical transit time of blood. Although renin and angiotensin have been reported in the renal interstitium and the lymph (17), where circulation time is not a critical factor, to date there have been no data to indicate that renal interstitial angiotensin (or for that matter extravascular angiotensin anywhere) can enter local arterioles to affect local hemodynamics.

Therefore, in the context of intravascular formation and effect alone, the time factor and the enzymatic kinetics of the renin-renin substrate reaction, as well as the singular property of angiotensin to constrict arteries in preference to veins, become important issues relative to any potential role for angiotensin as an intrarenal hormone. On the basis of these analyses, medullary formation and medullary vascular sites of action for intrarenal angiotensin seem reasonable. Present results in the isolated kidney support this hypothesis by demonstrating changes in the fractional blood flow to the inner cortex (and thus the medulla) associated with altered concentrations of renin substrate in the perfusate. These data based on the use of radioactive labeled microspheres are consistent with previous studies in which the 133Xe washout method was used to correlate changes in intrarenal blood flow with concentrations of renin substrate (2). Presumably we had established a critical level of renin substrate in the isolated preparation at which the ability of local renin to generate angiotensin at local vascular sites within the kidney was enhanced or depressed by respective increases or decreases in the concentration of renin substrate presented to it. Under more usual physiological circumstances in vivo, similar alterations in intrarenal angiotensin and medullary vascular resistance might be accomplished in response to changes in local juxtamedullary rates of renin secretion. Recent studies by Brown et al. (18) and Gavras et al. (19) indicated that juxtamedullary (inner cortical) concentrations of renin might be very sensitive to physiological stimuli. Although inner cortical concentrations of renin were usually less than those of the outer cortex, the relative changes in concentration in the juxtamedullary zone were often greater secondary to alterations in sodium metabolism or perfusion pressure. These

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observations and mechanisms might explain the published findings of other investigators (20) that salt loading increases medullary flow possibly by diminishing the local juxtamedullary secretion of renin. Our results are also consistent with the thesis of Thurau (21) and Thurau and Levine (22) that angiotensin might function as an intrarenal hormone to regulate the glomerular filtration rate and the distribution of blood flow within the kidney. We differ from Thurau in that our data indicate a medullary efferent arteriolar effect for intrarenal angiotensin, whereas Thurau proposed a cortical afferent effect. However, we are unaware of any study to date in which Thurau or his co-workers correlated their data with measurements of outer or inner cortical blood flow.

In contrast to intrarenal renin and angiotensin formed subsequently within the kidney, circulating renin and angiotensin should have a less selective renal site of action. Thus, in the present study, infusions of angiotensin II did not cause preferential reductions in medullary flow. It might be expected then, in accordance with previous studies (23, 24), that high plasma concentrations of either renin or angiotensin (extrarenal) would not correlate with reduced medullary flow. Possibly an additional reason for a lack of correlation between reduced medullary flow and circulating renin in plasma is that most circulating renin is not derived from the inner renal cortex but from the middle cortex and the outer cortex, which compose a major portion of the renal mass, have a greater quantity of renin, and receive by far the largest percent of the total renal blood flow. These thoughts and present results lead us to suggest that inner and outer cortical renin serve different physiological roles: the former relates more to intrarenal hemodynamics and the latter to more generalized body functions.

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References


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