Intrarenal Formation of Angiotensin I

Abstract. *In 5 patients and 16 dogs the mean concentration of angiotensin I, but not II, was higher in the plasma of the renal vein than in the plasma of the renal artery. The fact that I was higher in the vein than in the artery supports the concept that I is formed in the renal vasculature. In the vein the mean concentration of I was 45 times higher than that of II. The probable formation of angiotensin I within the kidney and its high concentration in the renal vein suggest that if the renin angiotensin system plays a role in the regulation of intrarenal blood flow, then angiotensin I may be the major effector hormone in this process.*

Circulating angiotensin II has important effects on renal function and blood flow in that it alters renal vascular resistance and influences systemic blood pressure and the secretion of aldosterone. Also the renin angiotensin system may have an additional role in the intrarenal distribution of blood flow by forming angiotensin within the kidney itself (*1*). This hypothesis has been advanced because renin is present at specific juxtaglomerular sites within the renal cortex and blood vessels (*2*). The localized release of renin at these sites could cause vasoconstriction of selective renal vessels and alter renal blood flow appropriately. Since renin has no vasoconstrictive effect of its own, but acts by way of angiotensin, a prerequisite of this theory is that angiotensin is formed within the renal vasculature as the blood flows through this organ. The demonstration that concentrations of angiotensin in the renal vein are higher than those in the renal artery would constitute evidence in support of the formation of angiotensin within renal blood vessels. Although higher renin levels have been demonstrated in renal venous blood, a similar relationship has not yet been established for angiotensin. Each of 16 dogs was anesthetized (pentobarbital, 65 mg/kg), and the dogs were bled (400 to 600 ml). Within 10 minutes, paired specimens of renal arterial and renal venous blood were drawn from each animal. This procedure stimulates a renin secretory response. Additional specimens of renal blood were obtained, during the course of routine diagnostic studies, from five human hypertensive subjects with unilateral renal arterial abnormalities. The blood was analyzed for angiotensin I, a decapeptide, and angiotensin II, an octapeptide, by the radioimmunoassay technique described by Goodfriend, Ball, and Farley (*3*), except that the plasma specimens were boiled for 10 minutes to destroy further renin and angiotensinase activity and only 0.1 ml of the supernatant was used in each assay tube. As a result of these differences our measurements of concentrations of angiotensin I and II in the plasma are higher than those reported previously, but nevertheless they have been consistent and reproducible.

Concentrations of angiotensin I in the plasma were consistently higher in the renal vein than in the renal artery in both the dog and the human. In contrast, we measured no significant elevation of angiotensin II in renal venous blood. The mean concentrations of angiotensin I (in the dogs) were 24 and 45 times greater than those of angiotensin II in renal arteries and renal veins, respectively. Because our antibody for angiotensin II cross-reacted with angiotensin I (approximately 1 percent cross-reactivity), our measured values for angiotensin II are falsely elevated, particularly in the renal vein where angiotensin I was especially high. Therefore our failure to demonstrate higher concentrations of angiotensin II in renal venous blood is even more significant than indicated by the data in Table 1. The higher concentrations of angiotensin I relative to angiotensin II in arterial blood is surprising in view of studies of others indicating a high rate of conversion of the decapeptide to the octapeptide during passage through the lung (*4*). We have no explanation for this. However, our measurements are based on simultaneous immunoassays for angiotensin I and II which have not been used previously in studies of pulmonary conversion.

Table 1. Concentration (nanograms) of angiotensin I and angiotensin II in 0.1 ml of plasma; R, right kidney; L, left kidney.

Subject		Angiotensin I		Angiotensin II	
		Renal artery	Renal vein	Renal artery	Renal vein
			Dog		
1		2.50	4.60	0.04	0.04
2		6.30	6.00	0.27	0.03
3		1.72	2.30	0.07	0.08
4		1.59	2.40	0.07	0.08
5		1.72	4.80	0.08	0.08
6		5.80	7.80	0.13	0.13
7		0.90	1.86	0.04	0.06
8		1.20	10.80	0.06	0.09
9		0.74	1.39	0.07	0.06
10		1.86	1.72	0.13	0.08
11		1.49	1.77	0.10	0.11
12		1.27	2.60	0.13	0.13
13		1.99	4.18	0.11	0.12
14		2.15	4.80	0.18	0.16
15		1.67	5.15	0.09	0.14
16		8.10	10.02	0.17	0.17
Mean ± S.E.		2.56 ± .54	4.51 ± .73	0.11 ± .01	0.10 ± .01
		$P < .02$		$P < .40$	
			Human		
K.C.		3.30	5.50	0.09	0.08
G.C.	L	2.10	1.30	0.21	0.20
	R	2.10	1.70	0.21	0.24
P.D.	L	5.70	9.60	0.18	0.18
	R	6.30	7.00	0.27	0.27
S.M.		4.10	5.05	0.46	0.51
D.A.		1.35	2.35		

Our results are consistent with the possibility that the enzymatic rate of reaction of the renin angiotensin system is rapid enough to produce angiotensin I within the renal vasculature. Although our data do not rule out the formation of small quantities of angiotensin II within renal blood vessels, this cannot be very significant since we were not able to demonstrate higher renal concentrations in the veins than in the arteries even after giving a hemorrhagic stimulus. The latter result is in agreement with reports of others (*5*) and probably indicates a lack of converting enzyme in renal blood (*4*). Angiotensin II has been measured in high concentration in renal lymph (*5*), but the physiologic significance of this finding is unknown, especially since angiotensin II at this location would have no selective effect on renal blood vessels. Increasingly, evidence indicates that angiotensin I has physiologic activity similar to that of angiotensin II although the decapeptide is only 2 to 5 percent as active as the latter (*6*). Since we demonstrated that renal venous concentrations of angiotensin I are approximately 50 times higher than those of angiotensin II, the differential quantities of these hormones appear sufficiently great to consider angiotensin I rather than angiotensin II as the major intrarenal vasoconstrictor.

Our studies support the formation of high concentrations of angiotensin I, but not angiotensin II, within renal blood vessels. If the renin angiotensin system plays a role in the intrarenal distribution of blood flow, angiotensin I may be the effector hormone.

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Uroporphyrinogen III Cosynthetase Activity in the Fox Squirrel (Sciurus niger)

Abstract. *The activity of uroporphyrinogen III cosynthetase in hemolyzates and tissue extracts from fox squirrels is much less than in similar preparations from gray squirrels. Low activity of this enzyme explains the production of large amounts of uroporphyrin I by the fox squirrel. Members of this species thus provide a small-animal model for studies of congenital erythropoietic porphyria, a hereditary disease of man and cattle which is associated with a similar partial deficiency of uroporphyrinogen III cosynthetase.*

The bones of the fox squirrel (*Sciurus niger*) are pink. In 1937, Turner (*1*) identified the skeletal chromophore as uroporphyrin I and demonstrated a porphyrinuria in this species. The formation, storage, and excretion of uroporphyrin I by healthy fox squirrels resemble manifestations of congenital erythropoietic porphyria, a hereditary disease of man and cattle (*2*). In this disorder, the overproduction of uroporphyrin I results from a partial deficiency of the enzyme uroporphyrinogen III cosynthetase (*3*). The data presented below show that the activity of this enzyme is very low in blood and other tissues from fox squirrels, which suggests that the physiological production of uroporphyrin I by this animal is on the same basis as its pathological production by porphyric men and cattle.

Uroporphyrinogen III cosynthetase in crude tissue extracts can be assayed by measuring the isomer composition of the uroporphyrinogen formed from porphobilinogen by partially purified uroporphyrinogen I synthetase (*3*). When the amount of cosynthetase added is appropriately limiting, the product is a mixture of uroporphyrinogens I and III, and the percentage of III is proportional to the amount of cosynthetase added (*4*). One unit of cosynthetase is defined as the amount that produces 50 percent isomer III, in the presence of enough synthetase to catalyze the formation of 5 nmole of uroporphyrinogen in 30 minutes at 31°C (*3*).

With this assay, uroporphyrinogen III cosynthetase has been assayed in blood samples from seven fox squirrels and 14 gray squirrels (*Sciurus carolinensis*). The fox squirrels were trapped on the Delmarva peninsula or in the mountains of western Maryland and the gray squirrels in the vicinity of Washington, D.C. Heparinized blood was obtained by cardiac puncture performed under methoxyfluorane anesthesia and was frozen in solid CO_2. Cosynthetase assays were carried out on thawed hemolyzates diluted with appropriate volumes of 0.05*M* potassium phosphate buffer (*p*H 7.9) as described previously for bovine blood (*3*).

The results of a typical experiment, in which graded amounts of 25-fold dilutions of squirrel hemolyzates were added to the standard reaction mixture containing porphobilinogen and synthetase (*3*), are shown in Fig. 1. Activity in hemolyzates from the 14 gray squirrels ranged from 3660 to 15,300 units per gram of hemoglobin, with a mean of 8900 units; this is almost 2½ times the mean previously found for normal human subjects (*5*). In contrast, cosynthetase activity was not measurable in 25-fold dilutions of the fox squirrel hemolyzates, nor on reassay in which threefold dilutions were used. Dilutions of squirrel blood less than threefold could not be assayed for cosynthetase activity because of technical difficulties in the assay procedure. The data indicated that cosynthetase activity in the blood of these seven fox squirrels was present at concentrations of less than 300 to 500 units per gram of hemoglobin, or less than 6 percent of the mean activity in the blood of gray squirrels. This amount of cosynthetase is in the range of the activity of blood from human and bovine subjects with congenital erythropoietic porphyria (*3*, *5*).

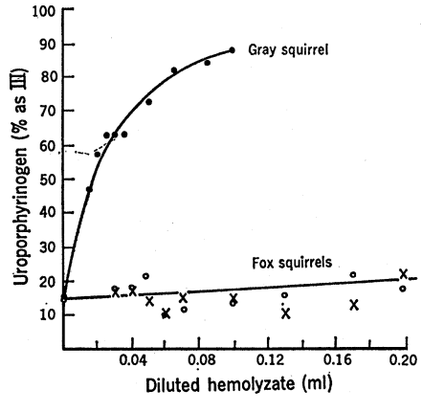

Fig. 1. A typical experiment demonstrating the measurement of uroporphyrinogen III cosynthetase activity in hemolyzates (diluted 25 times) from three squirrels. Closed circles, gray squirrel; open circles, fox squirrel No. 1; crosses, fox squirrel No. 2.

Hemolyzates from fox squirrels did not interfere with cosynthetase activity of hemolyzates from gray squirrels or humans, or the cosynthetase activity of mouse spleen extracts (*4*). This indicates that the low cosynthetase activity in fox squirrel blood is not due to excessive activity of enzyme systems that selectively remove uroporphyrinogen III while allowing uroporphyrinogen I to accumulate. The amounts of hemolyzate tested for cosynthetase activity did not contain significant uroporphyrinogen I synthetase activity under the assay conditions.

It is possible that uroporphyrinogen III cosynthetase activity varies from one species to another because of differences in the life span of red cells, since cosynthetase activity declines as the red cells age (*5*). However, it is unlikely that the red cell life span could differ enough between *S. niger* and *S. carolinensis* to account for the enormous difference in cosynthetase activity which has been observed. Routine hematologic indices were comparable in those individuals of the two species on which the cosynthetase assays were done. Hemoglobin values on the hemolyzates ranged from 10.6 to 16.4 g/100 ml, except for one gray squirrel that had a hemoglobin concentration of 5.3 g/100 ml. Reticulocyte counts in both species were 0.3 to 4.2 percent, except for three gray squirrels that had counts of 0.0, 5.4, and 7.5 percent,