Rate of Disappearance of Exogenous Dog Renin from the Plasma of Nephrectomized Dogs* (32678)

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The half-time of renin has been reported to be about 10–30 min (1–4). However, the conclusions in most previous studies have been based on only one or a small number of samples and in most cases the methods used for the assay of renin-like activity were not as sensitive as those now available. Renin-like activity has been reported to persist in the circulation 24 hours or longer after the removal of the kidneys (5–7), a finding which suggests that renin may have a much longer half-time. Therefore, we reinvestigated the problem of the half-time of renin in the dog. Exogenous dog renin was administered to nephrectomized dogs and the disappearance of this renin followed over a 3-hour period. Part of the data obtained has previously been reported in an abstract (8).

Methods. Animal procedures. Male mongrel dogs weighing 9–19 kg were anesthetized with pentobarbital sodium (30 mg/kg). A femoral artery and vein were cannulated and a control arterial blood sample was taken within 30 min after administration of the anesthetic. Both kidneys were removed and an additional blood sample collected 4 hours later. Twenty or 40 mg of a dog renin extract prepared by the method of Haas and Goldblatt (9) was then administered intravenously and arterial blood samples were withdrawn 2,5,10,15,30, 60, 90, 120, and 180 min after injection. After each collection an equal volume of donor blood from a nephrectomized dog was infused. Blood pressure was measured continuously by means of a Grass model-5 polygraph and a Statham strain gauge.

Renin assay. Blood samples were collected in graduated tubes containing ethylendiamine tetraacetic acid (EDTA) as anticoagulant (1 ml of a 3.8% solution per 10 ml of blood). The tubes were immersed in ice during the collection period and then immediately centrifuged at 0–2° C for 20 min at approximately 2000 rpm. The hematocrit was recorded and the plasma separated and stored at —20° C until extracted. The extraction method used was that of Boucher *et al.* (10) with certain modifications (11–13): (1) the pH of the incubation mixture was adjusted to 5.5 after the addition of resin to the plasma aliquot; (2) the incubation mixture was transferred to chromatographic columns and washed with 15 ml ammonium acetate buffer (pH 6), 20 ml of 10% (v/v) acetic acid and 15 ml of distilled water; and (3) the final product after sublimation was taken up into 0.9% saline containing 100 mg polyvinylpyrrolidinone (PVP)/100 ml.

The pressor activity of these extracts was assayed in 150–275 gm male Long-Evans rats which had been bilaterally nephrectomized approximately 18 hours previously. The rats were anesthetized with pentobarbital sodium (40 mg/kg) or urethane (1 gm/kg) supplemented as necessary with pentobarbital sodium. Two polyethylene catheters were inserted into one jugular vein for the administration of standard and sample. The carotid artery on the opposite side was also cannulated and the rats were then given pentolinium tartrate (25 mg/kg). Blood pressure was monitored by means of a Grass model-7 polygraph and a Statham strain gauge. Angiotensin II standard (1-L-asparaginyl-5-L-valyl angiotensin octapeptide, Hypertensin, Ciba, 0.5 μg/ml) was prepared in 0.9% saline containing 100 mg PVP/100 ml and divided into aliquots sufficient for one day's use. The aliquots were frozen and stored in siliconized

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tubes for up to 1 month. Both the standard solution and the samples were administered to the assay rats via microsyringes (Hamilton Co., Whittier, California). The volume of standard or sample injected usually ranged from 2–20 μl and never exceeded 100 μl. Only rats responding to 2 mμg of angiotensin II with at least a 10-mm Hg rise in mean blood pressure were used.

The increase in mean blood pressure of the assay rat is proportional to the logarithm of the dose of standard angiotensin II between 1 and 10 mμg (14, unpublished observations). Therefore, the assays were carried out and the potency of the unknown samples calculated using the four-point assay design of Schild (15). This permits the accurate measurement of angiotensin II levels as low as 0.5 mμg/ml of plasma.

To determine how much of the pressor activity was due to angiotensin and other pressor substances present in the plasma before incubation, measurements were carried out with the incubation step omitted. After adjusting the pH to 5.5, the plasma was added directly to a chromatographic column containing 5 ml of resin. Substrate levels were evaluated by comparing angiotensin II formation after 1 and 3 hours of incubation.

The recovery of angiotensin II (5–20 mμg/ml) added to plasma immediately after adding the resin and adjusting the pH was 88 $\pm$ 3% (mean $\pm$ SE) in 17 experiments. The coefficient of variation (standard deviation/mean) in four groups of 3–7 multiplicate samples was 14 $\pm$ 2%. In this paper, the renin values, unless otherwise noted, are the total mμg of angiotensin II/ml of plasma after a 3-hour incubation period. Means, when presented in the text, are accompanied by the standard error of that mean.

Analysis of data. A semilogarithmic plot of the renin values after renin injection versus time was made for each animal. The control value 4 hours after nephrectomy was subtracted from all subsequent values before they were plotted; in two dogs in which the control value was missing the average control value for the remaining six dogs was substituted. The data plotted in this manner showed two distinct slopes. The slope of the second component was calculated by the method of least squares using the experimental values for the last three sampling periods (generally 90, 120, and 180 min). This line was extrapolated to zero and substracted from the earlier points. The first three points were then used to calculate the slope for the first component.

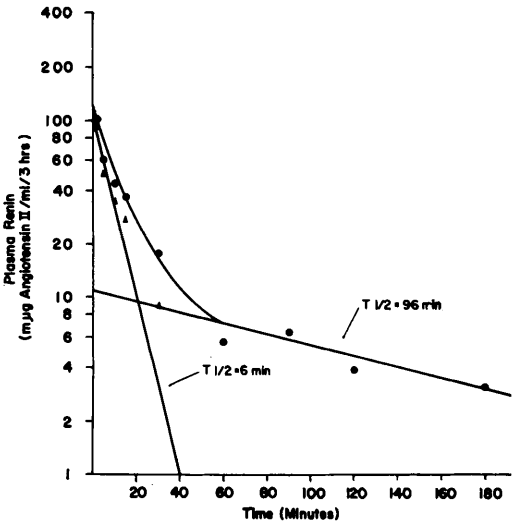

FIG. 1. Plasma renin levels after the administration of 20 mg (approximately 1 GU) of a renin preparation to a nephrectomized dog. The circles represent renin levels minus the control plasma level before renin injection, and the triangles the calculated values for the first component (see text). The straight lines are the slopes of the two components. $T\frac{1}{2}$, half-time.

Results. The mean plasma renin level in eight anesthetized dogs with intact kidneys was 10.2 $\pm$ 1.0 mμg/ml. Four hours after nephrectomy, the mean renin level was 1.3 $\pm$ 0.2 mμg/ml (six dogs); this value is significantly lower than the level before nephrectomy ($p < .001$).

A typical curve obtained after the injection of 20 mg of the dog renin extract is show in Fig. 1. The disappearance rate of renin from the plasma of dogs receiving 20 mg of the renin preparation did not differ significantly from the rate in those receiving 40 mg (Table I). The mean half-time of the rapid phase of renin disappearance in all the nephrectomized dogs was 6 $\pm$ 1 min; the slower phase had an average half-time of 79 $\pm$ 7 min.

In two dogs receiving 40 mg of renin extract, the pressor activity without incubation

TABLE I. Renin Levels in Plasma Following Injection of a Dog Renin Preparation in Nephrectomized Dogs.

		Mg of renin preparation injected	
	(Min)	20	40
Renin levels	Control	1.4 ± 0.3	1.3 ± 0.3
(mμg angiotensin II/ml/3 hours)	2	117.6 ± 6.1	198.3 ± 22.0
	5	70.5 ± 5.2	145.4 ± 20.0
	10	49.8 ± 2.8	100.3 ± 13.3
	15	46.3 ± 3.5	83.4 ± 8.5
	30	25.9 ± 6.1	45.5 ± 5.6
	60	19.5 ± 6.0	29.1 ± 3.3
	90	12.9 ± 2.6	21.3 ± 2.5
	120	8.8 ± 2.6	14.0 ± 0.9
	180	5.8 ± 1.1	10.8 ± 1.7
Half-time (min)	First component	5 ± 1	7 ± 1
	Second component	72 ± 14	83 ± 9

(i.e., the angiotensin present before incubation and the small amounts of other pressor substances that may enter the final extract) was measured 2,10,30,60, and 120 min after injection of renin. The average values obtained were 24.3, 13.0, 6.4, 3.2, and 2.7 mμg/ml. The curve formed from these values was subtracted from the renin disappearance curve and the half-time of the two components again calculated. The half-time of the first component in both dogs remained unchanged; the half-times of the second component were 69 and 104 min before subtraction and 68 and 98 min after subtraction of circulating angiotensin levels.

Substrate levels were assessed at 2, 15, 60, 90, and 120 min after injection in one dog receiving 40 mg of renin. Angiotensin-II formation is a linear function of the incubation time if substrate levels are not rate limiting. So during a 3-hour incubation period, three times as much angiotensin II should theoretically be formed as is formed during a 1-hour incubation period. The actual results are compared to the theoretical values in Table II.

The maximum increment in blood pressure following administration of the renin preparation occurred in 3–5 min. The increment averaged 53 ± 13 mm Hg systolic and 50 ± 10 mm Hg diastolic in the dogs given 20 mg of the renin preparation, and 64 ± 12 mm Hg systolic and 60 ± 6 mm Hg diastolic in dogs given 40 mg. In four dogs, the blood pressure returned to preinjection levels by 120 min; in five it returned by 180 min. In two dogs, it was still 20–25 mm Hg above preinjection levels at the end of 3 hours. In the remaining dog, blood pressure measurements were inaccurate and had to be discarded.

The total amount of angiotensin II generated per milliliter of plasma in each dog can be estimated by extrapolating the slope of the second component of the renin disappearance curve to the vertical axis. The mean of the values calculated in this way was 28.9 ± 9.0 mμg in the dogs given 20 mg of the renin preparation and 44.2 ± 6.6 mμg in the dogs given 40 mg. If one assumes that

TABLE II. Assessment of Angiotensinogen Levels.

Values were obtained after injection of 40 mg of the dog renin preparation.

	Angiotensin formation (mμg/ml)		
Time after injection (min)	1-hour incubation	3-hour incubation	Theoretical value[a] after 3-hour incubation
2	71.4	230.7	214.2
15	26.5	68.7	79.5
60	10.7	24.7	32.1
90	6.8	16.7	20.4
120	3.0	9.4	9.0

[a] 1-hour value × 3.

all the renin stays in the vascular system, the values for angiotensin II formation per 0.001 Goldblatt Unit (GU) of renin can then be calculated by multiplying the values for angiotensin II generated per milliliter plasma by the assumed plasma volume (5% of body weight) and dividing this value by the total number of units of renin injected, assuming that 20 mg of the renin preparation equals 1 GU of renin. This seems to be a reasonable assumption, since 20 mg of this preparation caused a mean systolic blood pressure rise of 30 ± 5 mm Hg upon injection into three pentobarbital-anesthetized dogs with intact kidneys. The Goldblatt Unit is the amount of renin that increases systolic blood pressure 30 mm Hg in unanesthetized dogs (1), but in our laboratory, 1 GU of renin also produces a rise of this magnitude in pentobarbital-anesthetized dogs (16). The calculated values for angiotensin II generated per 0.001 GU are 21.3 ± 8.6 mμg for the dogs receiving 20 mg of the renin preparation, and 12.7 ± 1.4 mμg for the dogs receiving 40 mg, or 15.9 ± 3.3 mμg for all the dogs combined.

Discussion. The method used in these studies measures the angiotensin generated during the 3-hour incubation period plus any angiotensin present at the start of incubation and the small amounts of other pressor substances that may be present in the final extract. Our data indicate that these latter two components make up approximately 15% of the total pressor activity. However, the percent is the same at high and low levels of circulating renin. Consequently, subtracting the values for pressor activity present before incubation from the values after incubation did not significantly change the slope of the disappearance curve. Thus, the curves accurately mirror renin disappearance.

The rapid component of the disappearance curve is probably due to mixing of the injected renin in the circulation. The reported mixing time for P^{32}-tagged red cells is 20.5 min and for T-1824 is 14 min in pentobarbital-anesthetized dogs (17). The mean half-time of the second component (79 ± 7 min) is somewhat longer than the previously reported values for the half-time of renin (1–4). In all the previous studies, relatively few measurements of circulating renin were made and no attempt was made to resolve the curves obtained into various components. Consequently, the slow component may have been missed.

The present studies also suggest that the half-time of endogenous renin in the circulation is similar to that of exogenous renin. Following removal of the kidneys, circulating renin levels fall in 4 hours from 10.2 to 1.3 mμg/ml. If the half-time were 80 min, the value at 4 hours would be 1.28 mμg/ml.

Some investigators have reported that renin is still detectable in the plasma 24 or more hours after nephrectomy (5–7). This raises the possibility that some of the renin is very slowly metabolized, but this possibility seems unlikely in view of the report of renin in the circulation many days after nephrectomy (5). If the substance being measured is actually renin rather than some other pressor substance, the renin may be entering the circulation from extrarenal sources. Such extrarenal sources have been reported (18).

The abundance of substrate in the one dog in which its concentration was assessed indicates that substrate concentration is not a regulating factor in the present experiments. The actual amount of angiotensin generated in 3 hours was almost equal to the calculated amount (Table II) and even these small differences disappear if estimated values for circulating angiotensin II are subtracted from the 1- and 3-hour renin values. Angiotensinogen has been reported to be present in excess in normal dogs (19) and nephrectomized dogs are said to have increased amounts of angiotensinogen (20).

Summary. Exogenous dog renin was administered intravenously to nephrectomized dogs and its disappearance followed for 3 hours. There were two exponential components to the disappearance curves, a rapid component with an average half-time of 6 ± 1 min and a slower component with an average half-time of 79 ± 7 min. Circulating angiotensin levels were shown to have no effect on either half-time value and the presence of adequate substrate was demonstrated. The mean plasma renin level in pentobarbital-anesthetized dogs was found to be 10.2 ±

1.0 mμg angiotensin II generated/ml/3 hours. Four hours after nephrectomy, it was 1.3 ± 0.2 mμg. This suggests that the half-time of endogenous renin is similar to that of exogenous renin.

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The Effect of Unilateral Vagotomy on Gastric Secretion in the Pylorus Ligated Rat* (32679)

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Acute unilateral vagotomy was found by Shay *et al.* (1) to slightly reduce the rate of gastric secretion in the pylorus ligated rat, whereas bilateral vagotomy markedly reduced both the rate of secretion and the acid concentration. In contrast, in the dog, Sebus and Charbon (2) reported that the gastric secretory response to insulin hypoglycemia and to histamine was not altered by cutting 75% of the vagal fibers to the stomach. In man, it is generally believed that incomplete vagotomy is a cause of recurrent peptic ulceration, and is associated with a positive gastric secretory response to insulin hypoglycemia.

It is possible that the small reduction in the rate of secretion after acute unilateral vagotomy in the rat (1) was simply due to excessive handling of the glandular stomach and other abdominal organs at the time of nerve section. We have attempted to repeat the experiments of Shay *et al.*, performing unilateral vagotomy in some rats one week prior to pyloric ligation, and in others at the time of pyloric ligation.

Methods. Wistar rats, from 150–230 gm, were randomly allocated to four groups. Gastric secretion was collected by the method of Shay *et al.* (3). After fasting for 48 hours in individual cages with free access to water, pyloric ligation was performed under light ether anesthesia, and the stomach was washed out with 0.15 *M* NaCl through a soft rubber catheter passed orally. Four hours later the animals were reanesthetized with ether, and a

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