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Distribution and Disappearance Rate of Submaxillary Renin[1] (39054)

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Previous investigations of the disappearance of renin from the vascular space have dealt exclusively with the halflife and distribution of renal renin (1–8). In those studies renin was measured as renin activity which may not represent true renin levels since it depends on substrate concentration and other factors. The submaxillary glands of adult male mice contain large amounts of renin (9–11) which we have recently obtained in pure form (12). The physiologic role, however, of this renin has not been clarified (13, 14), and no studies have been reported on its distribution and metabolism.

In the present study we utilized pure submaxillary gland renin (12) to investigate its distribution and disappearance rate and the the results were analyzed on the basis of a two-compartment system.

Materials and methods. Submaxillary gland renin. The purification of renin from mouse submaxillary glands was performed as described in detail in a previous report (12). In brief, after extraction in water, ammonium sulfate fractionation, and five steps of column chromatography on Sephadex G-100, diethylaminoethyl cellulose and carboxymethyl cellulose, a major fraction was obtained containing approximately 80% of the total submaxillary gland renin activity. This highly purified submaxillary renin (SR) was used in the present study.

Production of renin antibody. One mg of pure renin was mixed with Freud's adjuvant and was injected into the foot pads of a rabbit each week. One week after the fourth injection a highly titered antiserum to SR was obtained (15).

Preparation of labeled renin. ^{125}I-labeled SR was prepared using the chloramine-T method of Hunter and Greenwood (16). Five micrograms of SR was reacted with 1 to 2 mCi of ^{125}I (Cambridge Nuclear, Boston, Massachusetts) for 15 sec. The labeled renin was then separated on a column of Sephadex G-75. The specific activity of the radioactive renin was 100–200 mCi/mg.

Experimental procedures. Swiss albino male mice, weighing 35–40 g, were anesthetized with pentobarbital (60 mg/kg ip). The submaxillary glands were removed from all mice 24 hr prior to the experiment. For those experiments requiring mice whose submaxillary glands and kidneys were removed, nephrectomy was performed 2 hr prior to the experiment.

Fifty microliters of labeled SR solution (0.05 *M* phosphate buffer, pH 7.4) containing 4×10^5 cpm were injected into the right carotid artery of each mouse. Blood samples were collected via a catheter from the carotid artery 5, 10, 15, 20, 30, 45, 60, 90, and 120 min after injection of the labeled SR. At each time interval 50 μl of blood were drawn utilizing disposable micropipets and transferred into polypropylene tubes containing 0.5 ml of heparinized saline (100 units/ml). The samples were centrifuged in the cold and 0.4 ml of the obtained supernatant was transferred to polypropylene tubes and then 0.1 ml of antiserum (sufficient to bind 92% of the labeled SR) was added. The final volume of each tube was adjusted to 1.0 ml with 0.05 *M* phosphate buffer, pH 7.4, containing 0.5% bovine serum albumin and incubated for 3 days at 4°. At the end of incubation 0.1 ml of previously titered goat antirabbit gamma globulin antiserum and 0.1 ml of a 1:20 dilution of normal rabbit serum were added to each tube. These tubes were then incubated for 24 hr at 4° and centrifuged at 3000 rpm for 25 min. The resulting supernatant was separated from the precipitate and the radioactivity of each was counted in

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a well type gamma counter. Also, the radioactivity remaining in the original blood samples was counted in order to obtain the total radioactivity of each sample.

In order to study the uptake of labeled SR by various organs of the mouse, experiments were conducted in which mice were sacrificed by bleeding from the aorta 5, 10, 20, 30, and 60 min after the intravascular injection of labeled SR containing 4×10^5 cpm. The liver, kidneys, spleen, heart and lungs were removed and placed in 12 × 75 mm polypropylene tubes. The radioactivity in each organ was counted and expressed as a percentage of the total radioactivity injected.

Analysis of data. The radioactivity of the precipitate of the incubation tubes was considered as the amount of labeled SR remaining unmetabolized in the circulating blood. The total radioactivity of the 50 μl blood samples was represented by the sum of the radioactivity of the precipitate and supernatant in the incubation tubes, plus the radioactivity remaining in the original sample.

All radioactivities were expressed as a percentage of the radioactivity of the 5 min samples. When the radioactivities of the precipitates were plotted on semilogarithmic graph paper against time, the resulting disappearance curve was expressed as the sum of two exponentials. This was obtained by extending the linear portion of the curve to the axis and subtracting the values on this extrapolated line from the values on the original curve. The differences were plotted against time and a straight line was fitted by the method of least squares.

The general equation for this kind of disappearance curve is as follows:

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t}$$

where $C(t)$ is the radioactivity of the precipitate at time t, A and B are the y intercepts of the straight lines of the fast and slow components respectively, and α and β are their slopes. This biexponential nature of the disappearance curve is most simply explained on the basis of a two-compartmental distribution with bidirectional intercompartmental rate constants (Fig. 1). Intercompartmental rate constants are calculated as follows (17):

$$k_{10} = \frac{\alpha\beta\,(A + B)}{A\beta + B\alpha}$$

$$k_{12} = \frac{AB\,(\alpha - \beta)^2}{(A + B)\,(A\beta + B\alpha)}$$

$$k_{21} = \frac{A\beta + B\alpha}{A + B}$$

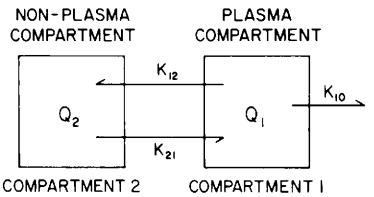

FIG. 1. Compartmental representation of the distribution of SR in the body with bidirectional intercompartmental rate constants.

Results. Uptake of labeled SR. The uptake of radioactivity following intravascular administration of labeled SR by various organs of the mouse is shown in Table I. Rapid accumulation of radioactivity occurred in the kidney. The percentage of the total dose of radioactivity found in the kidneys was 14.8 ± 1.6% (SE) in 5 min, 26.8 ± 0.7% (SE) in 10 min, 33.3 ± 1.9% (SE) in 20 min, 40.8 ± 0.3% (SE) in 30 min, and 42.6 ±

TABLE I. UPTAKE OF RADIOACTIVITY BY VARIOUS ORGANS OF MICE (n = 5). THE VALUES WERE EXPRESSED AS PERCENTAGE OF TOTAL RADIOACTIVITY (MEAN ± SE).

Organ	5 min	10 min	20 min	30 min	60 min
Kidney	14.8 ± 1.6	26.8 ± 0.7	33.3 ± 1.9	40.8 ± 0.3	42.6 ± 0.4
Liver	7.7 ± 0.7	8.0 ± 0.4	7.1 ± 0.5	6.3 ± 0.1	5.1 ± 0.2
Spleen	0.6	0.7	0.4	0.4	0.3
Heart	0.6	0.9	0.4	0.7	0.4
Lung	1.5	1.4	1.0	0.9	0.7

0.4% (SE) in 60 min. The percentage of uptake by the liver ranged from 5.1 to 8.0% throughout the experiment. The spleen, heart and lungs had only trace amounts of radioactivity at the various time intervals.

Disappearance of SR in sialoadenectomized mice. The disappearance curves obtained in sialoadenectomized mice were plotted on semilogarithmic graph paper and expressed as the sum of two exponentials. A typical curve is shown in Fig. 2. The mean half-times of the fast component and the slow component were 12.4 ± 0.4 min (SE) and 86 ± 3 min (SE), respectively. The calculated values of the rate constants k_{10}, k_{21}, and k_{12} based on the two-compartmental model were 0.0256 ± 0.0007 (SE), 0.0178 ± 0.0006 (SE) and 0.0207 ± 0.0012 (SE), respectively (Table II).

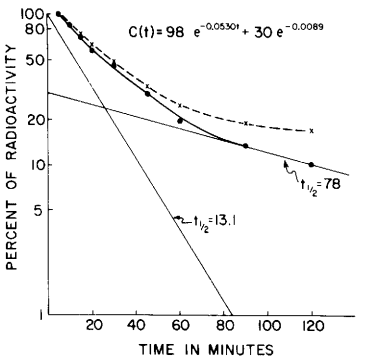

FIG. 2. Disappearance rate of labeled SR in nonnephrectomized mice. The crosses represent the total radioactivity of 50 μl of blood. The circles represent the radioactivity of the precipitate. The radioactivity of each sample is expressed as the percentage of the 5 min sample. The straight lines are the slopes of the two components.

Disappearance of SR in nephrectomized-sialoadenectomized mice. The disappearance curve of SR in nephrectomized–sialoadenectomized mice was also represented as the sum of the two exponentials. A representative curve is shown in Fig. 3. The mean half-time of the fast component was 14.7 ± 0.4 min (SE). The mean half-time of the slow component was 108 ± 7 min (SE), which was significantly longer ($P < 0.01$) than the slow component of nonnephrectomized mice. The values of k_{10}, k_{21}, and k_{12} were calculated to be 0.0115 ± 0.0009 (SE), 0.0280 ± 0.0011 (SE) and 0.0161 ± 0.0010 (SE) respectively (Table III). The values of k_{10} and k_{12} were significantly smaller ($P < 0.01$) than those of the nonnephrectomized mice. However, the value of k_{21} was significantly greater ($P < 0.01$) than that of the nonnephrectomized mice.

Discussion. There have been many reports on the disappearance rate of endogenous and exogenous renal renin in humans, dogs and rats. The half-time of the disappearance curve of renin in the circulating plasma, based on measuring plasma renin activity, has been reported as ranging from 10 to 280 min in various animals (1–8). In most of these studies, relatively few measurements of circulating renin were made. Some workers have shown that the disappearance curve of endogenous or partially purified homologous renal renin can be resolved into two exponential components (5–8).

TABLE II. DATA ON THE TWO-COMPARTMENTAL MODEL ANALYSIS OF LABELED SUBMAXILLARY RENIN DISAPPEARANCE FROM PERIPHERAL BLOOD IN SIALOADENECTOMIZED MICE.

Mouse no.	Fast component			Slow component			Rate constants		
	A%	$t_{1/2}$ min	α	B%	$t_{1/2}$ min	β	k_{10}	k_{21}	k_{12}
1	77.2	13.1	0.0530	22.8	78	0.0089	0.0249	0.0190	0.0180
2	80.7	14.1	0.0490	19.3	100	0.0069	0.0226	0.0151	0.0183
3	78.6	11.0	0.0630	21.4	82	0.0085	0.0267	0.0202	0.0247
4	81.3	11.5	0.0602	18.7	85	0.0082	0.0276	0.0179	0.0229
5	80.8	13.2	0.0525	19.2	74	0.0094	0.0279	0.0177	0.0163
6	81.2	11.7	0.0576	18.8	95	0.0071	0.0246	0.0166	0.0234
7	79.1	12.4	0.0560	20.8	89	0.0078	0.0246	0.0178	0.0214
Mean		12.4			86		0.0256	0.0178	0.0207
± SE		±0.4			±3		±0.0007	±0.0006	±0.0012

The present investigation is the first report on the disappearance curve of SR. The purified renin used in these experiments has been shown to resemble kidney renin in its enzymatic and biological activities (12). When labeled SR is injected intravascularly in the mice, the radioactive substances in the plasma are presumed to be labeled SR, radioactive metabolites, and free radioactive iodide.

As a result of incubating the plasma samples with excessive antibody to SR, the radioactivity of the precipitate might be considered to reflect the approximate amount of SR remaining unmetabolized in the circulation. Indeed, as shown in Figs. 2 and 3, there was a significant difference between the total radioactivity of the blood and the radioactivity of the precipitate in the late phase of the disappearance curves. This difference was probably due to radioactive metabolites and free radioactive iodide remaining in the circulation.

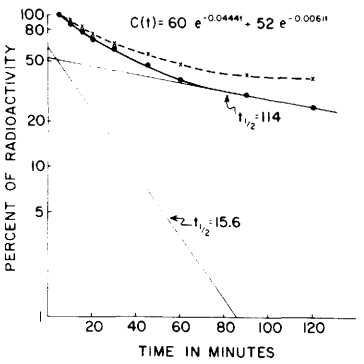

FIG. 3. Disappearance rate of labeled SR in nephrectomized mice. The crosses represent the total radioactivity of 50 μl of blood. The circles represent the radioactivity of the precipitate. The radioactivity of each sample is expressed as the percentage of the 5 min sample. The straight lines are the slopes of the two components.

Since it has not been established whether SR and renal renin have identical structures, the half-time values of SR obtained in our studies may not represent the half-time of renal renin. Thus, it is difficult to compare our results to those of previous reports on renal renin. Also, we used a tracer dose of labeled SR which caused no change in blood pressure, whereas in the previous reports a relatively large amount of partially purified renal renin was administered to various animals which induced a sustained increase in blood pressure.

In the present study, the fast component of the disappearance curve was probably due to equilibration of the injected labeled SR in the circulation. The reported mixing time for ^{32}P-tagged red cells was 20.5 min in pentobarbital-anesthetized dogs (18). A significant reciprocal relationship between the half-time of the fast component and the rate constant k_{12} seems to support the above concept (Fig. 4). However, as shown in Table I and Fig. 3, the fast component may be related to some extent to the rapid uptake of labeled SR by the kidneys. This, together with circulatory changes induced by nephrectomy, may explain the differences in half-times of the fast component in sialoadenectomized mice and nephrectomized–sialoadenectomized mice.

TABLE III. DATA ON THE TWO-COMPARTMENTAL MODEL ANALYSIS OF LABELED SUBMAXILLARY RENIN DISAPPEARANCE FROM PERIPHERAL BLOOD IN NEPHRECTOMIZED–SIALOADENECTOMIZED MICE.

Mouse no.	Fast component			Slow component			Rate constants		
	A%	$t_{1/2}$ min	α	B%	$t_{1/2}$ min	β	k_{10}	k_{21}	k_{12}
1	51.9	13.2	0.0521	48.1	135	0.0051	0.0095	0.0278	0.0197
2	53.5	15.6	0.0444	46.5	114	0.0061	0.0113	0.0239	0.0153
3	54.2	15.0	0.0461	45.8	83	0.0083	0.0149	0.0259	0.0138
4	57.2	13.2	0.0525	42.8	94	0.0074	0.0145	0.0267	0.0186
5	49.1	12.8	0.0541	50.8	102	0.0068	0.0119	0.0308	0.0181
6	40.7	15.1	0.0458	59.3	124	0.0056	0.0086	0.0294	0.0133
7	44.2	13.5	0.0514	55.8	103	0.0068	0.0101	0.0321	0.0140
Mean		14.7			108		0.0115	0.0280	0.0161
± SE		±0.4			±7		±0.0009	±0.0011	±0.0010

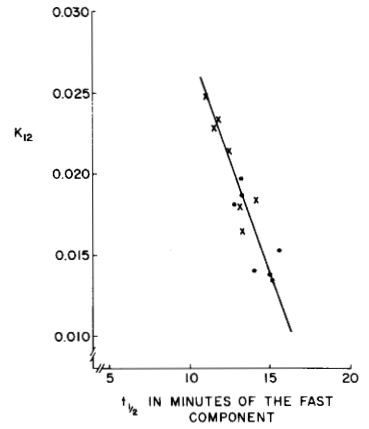

FIG. 4. Relationship between $t_{1/2}$ of the fast component of the SR disappearance curve and the rate constant k_{12} in nonnephrectomized mice (crosses) and in nephrectomized mice (circles).

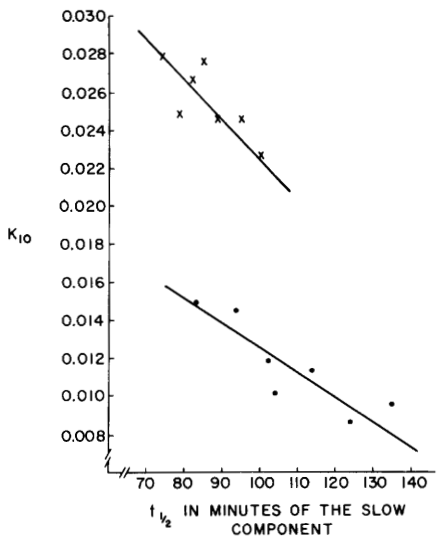

FIG. 5. Relationship between $t_{1/2}$ of the slow component of the SR disappearance curve and the metabolic rate constant k_{10} in nonnephrectomized mice (crosses) and nephrectomized mice (circles).

It seems reasonable to assume that the half-time of the slow component represents the true half-time of SR in mice. In fact, a significant reciprocal relationship between the half-times of the slow component and metabolic rate constant, k_{10}, was observed both in sialoadenectomized mice and in nephrectomized–sialoadenectomized mice (Fig. 5). The half-time of the slow component in nonnephrectomized mice was significantly shorter than the half-time of slow component in nephrectomized mice; thus, the kidney may be involved in the metabolism of SR.

Recently, Peters–Haefeli has found that the presence of the kidney contributes to the inactivation of exogenous homologous renin in rats and that this contribution depends on the total amount of renal tissue present (8). Similarly, in dogs, exogenous dog renin appears to be inactivated faster in the intact animals than in the nephrectomized animals (1, 7). Heacox *et al.* (19) reported, however, that the rate of inactivation in the dog liver was great enough to account for the inactivation observed in the whole animal. It was not possible in the present study to establish the role of the liver in the inactivation of SR. The amount of radioactivity found in the liver was much smaller than that found in the kidney; however, it is possible that the liver metabolizes the labeled SR and that the labeled metabolites are released by the liver and taken up by the kidney. This would, at least in part, account for the large amount of radioactivity observed in the kidneys.

Summary. Highly purified submaxillary renin (SR) labeled with ^{125}I was injected intravascularly into adult male mice following removal of submaxillary glands and kidneys, and the disappearance of this labeled SR from the circulating vascular volume was studied on the basis of a two compartment system. There was a fast and a slow component to the disappearance curves. Mean half-times of the fast and slow component were 12.4 $\pm$ 0.4 min and 86 $\pm$ 3 min in sialoadenectomized mice, while in mice whose submaxillary glands and kidneys were removed the half-times were 14.7 $\pm$ 0.4 min and 108 $\pm$ 7 min, respectively. The uptake of radioactivity by various organs of the mouse was also measured. Accumulation of radioactivity occurred in the kidneys and liver. Only trace amounts of radioactivity were found in the other organs.

The findings suggest that the fast component of the disappearance curve was probably due to equilibration of the injected labeled SR in the circulation. However, the fast component may be related to some extent to the rapid uptake of labeled SR by the kidneys. The half-time of the slow com-

ponent may represent the true halflife of SR in mice, since a significant reciprocal relationship between the half-times of the slow component and metabolic rate constant k_{10} was observed both in sialoadenectomized mice and in nephrectomized–sialoadenectomized mice.

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