Renal Blood Flow, Glomerular Filtration Rate, Renal PAH Extraction Ratio, and the Role of the Renal Vasomotor Nerves in the Unanesthetized Rabbit

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With the technical assistance of Yvonne Pascoe

The rabbit offers some advantages as an unanesthetized preparation over other species, in that it will remain quietly, without previous training, in a small cage during a variety of experimental procedures. Previous work from this laboratory has shown that the circulation of the rabbit studied under these conditions is stable as judged by the relative constancy of the resting cardiac output, blood pressure and heart rate over a period of hours. The variability of these measurements was similar to that observed in man or the dog. However, a number of workers have considered the renal circulation of the rabbit to be less stable than that of other species, and the rabbit has not been widely used for renal circulatory studies.

On the other hand, Forster, and Brod and Sirota obtained reasonable degrees of reproducibility of renal blood flow and glomerular filtration rate in unanesthetized lightly restrained rabbits. In its response to water loading the renal circulation of the rabbit is said to resemble that of marine mammals and amphibia and to differ from that of man and the dog.

During the course of an investigation on the role of the renal circulation in the circulatory adjustments to hypoxia, the opportunity presented itself to re-examine the question of the stability of the renal circulation of the rabbit, and to extend the range of normal measurements available for this species.

From the School of Physiology, University of New South Wales, Sydney, Australia.

Supported by a Grant-in-Aid from the National Heart Foundation of Australia.

Received for publication November 1, 1962.

Circulation Research, Volume XII, April 1963.

Methods

ANIMALS

The results were obtained from 86 batch-bred male rabbits of mixed breed varying in weight from 1.5 to 3.5 kg. The animals were kept on a diet of dry rabbit pellets, water ad libitum, and in addition they received greens on alternate days. No dietary restrictions were imposed before an experiment.

MEASUREMENT OF RENAL CLEARANCES AND CARDIAC OUTPUT

All animals had a preliminary operation using light sodium pentobarbital (Nembutal) anesthesia three to seven days before each experiment. The trachea was mobilized and placed into a more subcutaneous position in order to facilitate subsequent tracheotomy using local anesthesia, and to minimize accumulation of secretions in the tracheotomy tube during an experiment.

On the day of the experiment all operative procedures were carried out following infiltration of skin and subcutaneous tissue with 1% lidocaine HCl (Xylocaine), which does not interfere with the PAH reaction. Bladder catheterization was carried out using a No. 7 Jacques catheter, with the animal held by an assistant. The catheter was fixed in position by passing it through a small brass cylinder which was stitched to the skin of the perineum. The animal was then placed into a rabbit box and catheterization of the right atrium via the external jugular vein, followed by tracheotomy and insertion of a metal tracheal tube were carried out. A fine plastic catheter was inserted into the esophagus and passed into the stomach to allow giving water to the animal. The catheter was fixed in position by fine sutures through the esophageal wall.

The respiratory valve was
**FIGURE 1**

Results from unanesthetized rabbit showing the timing of three sets of determinations of ventilation, cardiac output, car artery pressure, renal blood flow (RBF) and glomerular filtration rate (GFR); also the timing of the infusion of PAH and creatinine, and of 5% mannitol in Ringer.

Period A-B = "control" period; C-D = first "treatment" period; E-F = second "treatment" period.

This was followed by a sustaining infusion (containing: creatinine 2.5 g; 20% para-amino hippurate 2.5 ml; Ringer-Locke solution to 80 ml) given at the rate of 0.18 ml/min by continuous intravenous infusion. The plasma levels obtained in different experiments using these solutions varied between 20-40 mg% for creatinine, and between 1.3 mg% for PAH.

Figure 1 illustrates the experimental design employed when testing stability of the preparation. The sustaining infusion of creatinine and PAH was given for 60 minutes before commencing clearance determinations. It was always necessary to augment the normal low urine flow in the rabbit by infusing 5% mannitol in Ringer-Locke solution at the rate of approximately 0.5 ml/min starting 15 minutes before each clearance schedule. Three urine collection periods of ten minutes each, and two arterial blood samples taken at the mid-point of the first and third period formed the basis of a "single estimate" of RBF and GFR (period A-B in fig. 1). The urine volume was noted after eight minutes. Bladder washings were carried out during the last two minutes of each collection period, with accurately measured amounts of normal saline; only very rarely was it necessary to introduce a few ml of air into the bladder to achieve quantitative return of the washings. Emptying the bladder by suprapubic pressure was avoided because it disturbed the animals.

RENAL VEIN CATHETERIZATION

Tracheal transposition was carried out in the usual manner. On the day of the experiment catheterization of the ear vessels was carried out using local anesthesia. The animal was then lightly anesthetised with sodium thiopentone (initial dose 18 mg/kg). Following local infiltration of the skin of the abdomen, a small paramedian incision was made exposing the right kidney and renal vein. After identifying the point of entry of the right renal vein into the inferior vena cava, a fine transflex catheter was inserted into the right external jugular vein, and passed into the inferior vena cava. The catheter was manipulated into the right renal vein so that its tip lay just to the vena caval side of the hilum. The abdominal wound was closed and the catheter was brought out through the neck and anchored...
RENAL CIRCULATION IN THE RABBIT

to the ear. The tracheal tube and oropharyngeal catheter were inserted in the usual way and the
rabbit recovered from the short-acting barbiturate
within a few minutes after completing the opera-
tion. The animal was placed into the experimental
cage and given 20 ml of warm tap water hourly
for two to three doses. Blank blood samples
were taken and priming and sustaining infusions
commenced three to four hours after recovery
from the anesthetic. The animals recovered quickly
from the operation and were alert and in no
apparent distress during the experiment. Arterial
and renal vein blood samples for creatinine and
PAH estimations were taken simultaneously dur-
ing the first and third urine collection periods
of each set of measurements. Samples for arterial
and renal vein O2 content and hemoglobin were
taken at the mid-point of the second collection
period. No cardiac output measurements were
made in these experiments.

CATHETERIZATION OF THE URETERS

In order to assess the effect of unilateral renal
denervation on the renal blood flow, separate
clearance measurements were made from each
kidney. After catheterization of the ear vessels,
the animals were lightly anesthetized with sodium
thiopentone. A midline lower abdominal incision
was made, and both ureters were identified close
to the bladder. The urine flow was augmented by
means of mannitol, and each ureter was cathe-
terized with plastic transflex catheters inserted
through a No. 14 Luer needle for a distance of
3 to 4 cm. The ureter and indwelling catheter
were sutured to the underlying psoas muscle,
and the catheters were brought cut through the
skin lateral to the psoas. After closing the abdomi-
nal wound and completing the neck operation
the animal was placed in the experimental cage
after it had recovered from the anesthetic. It
was necessary to maintain high urine flow (0.2
to 0.4 ml/min per kidney) by giving a continuous
infusion of 5% mannitol in Ringer-Locke solution.
In order to prevent dehydration the animals were
given 40 ml of water immediately after the
operation, followed by 20 ml of water every hour
for the next four hours. Priming and sustaining
infusions of creatinine and PAH were given at
the end of this period in the usual manner.

Figure 2 shows the results of a typical experi-
ment with both kidneys innervated. The RBF
and GFR were nearly equal on the two sides,
and small changes in these measurements on one
side were accompanied by similar changes on the
other side. When renal denervation was carried
out it was carried out using Nembutal anesthesia
and standard aseptic precautions six to seven
days before an experiment. In addition the ani-
mals were given 50 mg Terramycin orally twice
daily for two days following the operation. The
left kidney was always selected for denervation,
and all visible nerves lying in the renal pedicle
were cut, and the adventitia was stripped over
a distance of about 1 cm proximal to the hilum.

CREATININE AND PAH ESTIMATIONS

These were carried out on diluted urines and
on cadmium sulphate precipitates of plasma. The
amount of plasma required for each estimation
was 0.5 ml. Creatinine was determined using the
alkaline picrate method of Folin and Wu, and
PAH using the method of Smith et al. 
The
colorimetric measurements were made on a Beck-
man model DU spectrophotometer at a wavelength
of 520 mp for creatinine and 540 mp for PAH.

DISTRIBUTION OF PAH IN RABBIT BLOOD

It is known that some PAH enters the red
blood cells of the dog both in vivo and in vitro,
but not those of man. 
The recovery of known
amounts of PAH from rabbit blood was deter-
mimed in blood kept at 24°C or 38°C after
centrifuging at 15 cm radius at either 1800
rev/min or 3000 rev/min. When allowance had
been made for plasma trapping, recovery was
virtually complete (99%) from blood at either temperature centrifuged at 1800 rev/min. Recovery was somewhat less complete (94%) after centrifuging blood at 3000 rev/min. In this regard rabbit blood behaves more like human blood than dog blood in that PAH does not readily enter red cells. In vivo estimates of renal plasma flow by direct application of the Fick principle showed good agreement between simultaneous PAH and creatinine estimates in the rabbit; the mean value of RPF for 21 estimations was 54.2 ml/min using creatinine and 52.4 ml/min using PAH, this difference not being statistically significant.

**CARDIAC OUTPUT DETERMINATIONS**

These were carried out by the direct Fick method as described previously. Arterial and right atrial blood samples were analyzed for O₂ content by the micro-method of Roughton and Scholander, and hemoglobins were determined from each sample using the cyanmethemoglobin method of Drabkin and Austin. Air gas analyses were carried out using a Beckman model E 0₂ analyser for O₂, and the Haldane apparatus for CO₂ analyses.

**EAR ARTERY PRESSURE**

This was recorded using a Statham P 23 D strain gauge and a Sanborn recorder. The relationship between ear artery pressure and carotid pressure, breathing air and low oxygen mixtures has been previously described. Since many experiments using this preparation involved pressure determinations whilst breathing carbon monoxide, simultaneous femoral and ear artery pressures were determined in three animals whilst breathing 0.2% CO in air; it was found that, as with low O₂ mixtures, the ear artery pressure followed the pressure changes of the femoral artery, although the mean pressure of the ear artery was on an average 6 mm Hg below that in the femoral artery.

**Results**

The results in table 1 show that the values for resting cardiac output, arterial pressure, RBF and GFR remained reasonably constant in nine unanesthetized rabbits over a period of three hours. The standard error of a single replicate of RBF and GFR was approximately 8% of the respective mean values of these measurements. All experiments were carried out during moderate water and mannitol diuresis, since the spontaneous urine flow of the rabbit (0.05 to 0.2 ml/min) is too low to permit accurate determination of renal clearances. The hematocrit ratios observed during the
TABLE 2

Mean Values and Standard Deviations of the Control Observations, Obtained from 42 Completely Unanesthetized Rabbits (Group 1) and from 16 Rabbits Studied After Renal Vein Catheterization (Group 2)

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Group 1</th>
<th></th>
<th>Group 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>2.3</td>
<td>0.55</td>
<td>2.3</td>
<td>0.32</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>16.7</td>
<td>2.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac output (ml/min)</td>
<td>465</td>
<td>106.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean artery pressure (mm Hg)</td>
<td>91.1</td>
<td>7.75</td>
<td>85.9</td>
<td>3.97</td>
</tr>
<tr>
<td>Heart rate per min</td>
<td>262</td>
<td>33.3</td>
<td>267</td>
<td>27.3</td>
</tr>
<tr>
<td>Hematocrit (arterial) per cent</td>
<td>35.9</td>
<td>2.74</td>
<td>32.9</td>
<td>2.97</td>
</tr>
<tr>
<td>Urine flow (ml/min)</td>
<td>0.62</td>
<td>0.22</td>
<td>0.60</td>
<td>0.20</td>
</tr>
<tr>
<td>RBF (ml/min)</td>
<td>93.7</td>
<td>22.7</td>
<td>86.1</td>
<td>16.6</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>13.6</td>
<td>3.39</td>
<td>13.5</td>
<td>2.68</td>
</tr>
<tr>
<td>EPF (ml/min)</td>
<td>61.6</td>
<td>13.11</td>
<td>56.6</td>
<td>9.68</td>
</tr>
<tr>
<td>E, blood loss — per cent</td>
<td></td>
<td></td>
<td>95.5</td>
<td>2.84</td>
</tr>
<tr>
<td>Venilation (ml/min STPD)</td>
<td>24.0</td>
<td>5.85</td>
<td>24.5</td>
<td>3.42</td>
</tr>
<tr>
<td>Renal V O (ml/min STPD)</td>
<td></td>
<td></td>
<td>2.1</td>
<td>0.35</td>
</tr>
<tr>
<td>Sm, per cent</td>
<td>96.9</td>
<td>1.98</td>
<td>96.0</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rn, (High strain) per cent</td>
<td>57.5</td>
<td>7.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal vein O 2 per cent</td>
<td></td>
<td></td>
<td>81.4</td>
<td>3.0</td>
</tr>
<tr>
<td>Ventilation (ml/min STPD)</td>
<td>1358</td>
<td>292</td>
<td>1308</td>
<td>256</td>
</tr>
</tbody>
</table>

*Based on 20 measurements.
†Based on 32 measurements in group 1, and 13 in group 2.
§In group 1, EPF was estimated from uncorrected PAH clearances, but in group 2 estimated from (GFR/EI. AH)

12 observations only.

control period were somewhat lower than normally found in rabbits not receiving infusions. Hematocrit determinations were carried out in six animals before commencing infusions, and during the control clearance period; the mean changes in hematocrit were from 37.3% to 34.1%, the difference being statistically significant (P = 0.05). The results show that there is some blood volume expansion resulting in slight hemodilution as a result of the infusions. The additional fall in hematocrit ratio which was observed during the various treatment periods is probably largely due to blood sampling carried out in relation to the various test procedures.

Table 2 shows the mean values and standard deviations of the various measurements obtained from 42 unanesthetized rabbits during the control period (period A-B in fig. 1), and from 16 animals following renal vein catheterization. Comparison of the results of the latter group with those obtained from completely unanesthetized animals indicates that the operative procedures associated with renal vein catheterization were without significant effect on the renal circulation. The average value for the renal PAH extraction ratio was 95.5%, and was below 90% in only 1 out of 16 animals. In view of the high PAH extraction ratio in the rabbit the use of the uncorrected PAH clearance for estimating true renal plasma flow appears justified. The renal vein O 2 saturation was high in the rabbit, as in man, and the renal O 2 consumption averaged 8% to 10% of the total V O.
TABLE 3
Statistical Interrelationships of RBF With Body Weight, Kidney Weight, and Cardiac Output; Also Relationship Between GFR and RPF in Completely Unanesthetized Rabbits

<table>
<thead>
<tr>
<th>Number of rabbits</th>
<th>Regression equation</th>
<th>E^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>42</td>
<td>RBF = 33.3 + 37 BW</td>
<td>4.95</td>
</tr>
<tr>
<td>20</td>
<td>RBF = 30.5 + 3.95 KW</td>
<td>1.08</td>
</tr>
<tr>
<td>42</td>
<td>RBF = 32.8 + 0.151 C.O.</td>
<td>0.023</td>
</tr>
<tr>
<td>42</td>
<td>GFR = 4.29 + 0.292 RPF</td>
<td>0.028</td>
</tr>
</tbody>
</table>


^aStandard error of regression coefficient.

of the GFR to the renal plasma flow is also given in this table. The mean RBF was 23% of the mean cardiac output and the mean filtration fraction was 22% in the rabbit.

The contributions of nervous factors to the resting vasomotor tone were studied by comparing the RBF and GFR in one kidney with an intact nerve supply, with these measurements in the denervated kidney. Animals with both ureters catheterized had a more prolonged period of mannitol infusion and received larger quantities of water than the other groups. The hematocrit ratio of the 20 animals in this group during the control period was 36.6 ± 3.72 (SD), thus not differing significantly from the other groups. Table 4 shows that in eight control animals where both kidneys were innervated there was no significant difference in RBF and GFR on the two sides. In twelve animals where unilateral renal denervation had been carried out six to seven days previously the RBF and GFR were on the average 6% higher on the denervated side (P = 0.02). There was evidence of adequate denervation in these animals as judged by their response to severe arterial hypoxia.31

The results suggest that the amount of resting sympathetic constrictor tone exerted on the renal circulation of the rabbit is low.

Discussion

The results indicate that the unanesthetized rabbit is a satisfactory animal for studying the renal circulation. The RBF and GFR remained stable over a number of hours, as did the cardiac output, arterial pressure and heart rate. The mean RBF was 23% of the cardiac output, and the renal fraction thus being slightly greater in the rabbit than in man.3,10

The mean filtration fraction was 22% in the rabbit, closely similar to values observed in man, and somewhat below the average values reported for the dog.3 The demonstration of a low degree of sympathetic constrictor tone in the resting renal circulation of the rabbit is similar to the findings observed in man,23 and suggests that under the present experimental conditions the rabbit did not exhibit the degree of autonomie instability traditionally ascribed to this species.

The response of the renal circulation of the rabbit to large water loads has been considered one important species difference from other mammals. Kaplan and Smith,4 Dicker and Heller,7,8 and Forster,5 observed an increase in GFR, renal plasma flow, and glucose Tm after giving rabbits approximately 40 ml/kg of water by stomach tube for two to three half-hourly doses. This finding has been interpreted as indicating the recruitment of previously unperfused glomeruli, and resembles the behaviour of marine mammals and amphibia.9,10 Brod and Sirota8 have questioned this interpretation and consider that variations in the degree of excitement associated with the administration of these large amounts of water was an important factor in the renal vascular response. It is doubtful whether the response to such large water loads is of much physiological significance, since the animals received a total amount of water in the space of 60 to 90 minutes between two to three times their blood volume. The present experiments were not designed to test the effects of such large water loads, but demonstrate that during conditions of moderate water loading and mannitol diuresis the GFR of the rabbit was stable. The possibility that during these conditions the glomeruli of the rabbits were kept maximally open and functional cannot be ex-
Comparison of GFR (ml/min) and RBF (ml/min) on Left and Right Kidney in 20 Rabbits. In Group A Both Kidneys Innervated; in Group B Left Kidney Denervated. Each Measurement is Mean of 4 Clearance Periods

<table>
<thead>
<tr>
<th>Group</th>
<th>Left</th>
<th>Right</th>
<th>L/R</th>
<th>Left</th>
<th>Right</th>
<th>L/R</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7.3</td>
<td>7.4</td>
<td>0.99</td>
<td>47.6</td>
<td>46.0</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>7.1</td>
<td>7.3</td>
<td>0.97</td>
<td>25.0</td>
<td>27.8</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>9.7</td>
<td>7.9</td>
<td>1.10</td>
<td>57.9</td>
<td>37.1</td>
<td>1.02</td>
</tr>
<tr>
<td></td>
<td>8.4</td>
<td>7.3</td>
<td>1.09</td>
<td>45.7</td>
<td>46.8</td>
<td>0.97</td>
</tr>
<tr>
<td>B</td>
<td>3.1</td>
<td>3.6</td>
<td>0.86</td>
<td>25.7</td>
<td>27.1</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>6.1</td>
<td>6.3</td>
<td>0.97</td>
<td>42.5</td>
<td>43.9</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>7.3</td>
<td>7.1</td>
<td>1.03</td>
<td>45.8</td>
<td>45.9</td>
<td>1.06</td>
</tr>
<tr>
<td></td>
<td>8.5</td>
<td>9.2</td>
<td>0.93</td>
<td>57.0</td>
<td>62.0</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Mean: 0.94 7.02 0.99

Mean: 0.97 6.52 1.00

eluded. It seems however unlikely that the infusions of mannitol exerted significant pharmacological effects on the renal blood vessels, since relatively small degrees of vasoconstriction could be demonstrated in these animals during inhalation of carbon monoxide,\textsuperscript{11} and small differences in vasoconstrictor activity could be demonstrated as a result of renal denervation. The amount of osmotically active substances infused in the present experiments was considerably below that used in the experiments of Forster\textsuperscript{3} and Brod and Sirota.\textsuperscript{6} While the possibility thus remains that with very large water loads the renal circulation of the rabbit does differ from that of other mammals, this probably does not apply to more moderate water loads.

The results in table 2 for RBF and GFR are significantly higher than those reported previously by other workers.\textsuperscript{**} The values for cardiac output, arterial pressure and heart rate are similar to those reported in normal rabbits not receiving infusions.\textsuperscript{1} In the present experiments it was found to be particularly important to keep the animals in their experimental cages without restraint, to avoid handling the animals when collecting blood and urine samples, and to avoid overhydration by the administration of excessive water loads, or on the other hand dehydration by the prolonged use of strong osmotic agents. It seems likely that a greater degree of renal vasoconstriction was present in some of the previous series than in the present experiments, possibly as a result of the rabbit's smaller tolerance to external stimuli and to changes in fluid balance compared to larger animals. The results indicate that under appropriate experimental conditions the stability of the renal circulation of the rabbit is closely comparable to that of other species.

Summary

The unanesthetized rabbit has been found to be a satisfactory animal for studying the renal circulation. Techniques for measuring renal clearances and cardiac output, including renal vein catheterization, and for measuring renal clearances separately for each kidney are described. Normal values are pre-
sented from data obtained from 86 rabbits. Renal blood flow and glomerular filtration rate were found to be stable, judged by the reproducibility of these measurements over a period of three hours. Renal PAH extraction ratios averaged 95.5% in 16 unanesthetized animals. In 12 animals renal blood flow was 6% higher in one chronically denervated kidney than in the contralateral innervated kidney, indicating a low degree of sympathetic vasoconstriction in the normal resting circulation of the rabbit. All the results were obtained in the rabbit in the course of moderate water and mannitol diuresis.

References


Renal Blood Flow, Glomerular Filtration Rate, Renal PAH Extraction Ratio, and the Role of the Renal Vasomotor Nerves in the Unanesthetized Rabbit

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Circ Res. 1963;12:353-360
doi: 10.1161/01.RES.12.4.353

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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