Validation of 'transit renography' for the determination of the intrarenal distribution of plasma flow: comparison with the microsphere method in the anaesthetized rabbit and pig


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Summary

1. The spectrum of transit times of sodium o-iodohippurate (Hippuran) through the kidney can be derived from an 131I- (or 123I-) labelled Hippuran renogram by deconvolution. In the rabbit and pig, as has previously been shown in man, the frequency distribution curve for the transit times was bimodal. Since the transit time is likely to be proportional to the nephron length, the area of the first mode is likely to represent plasma flow to the shorter outer cortical nephrons whereas the delayed mode represents flow to the long juxtamedullary nephrons.

2. This interpretation was tested by simultaneously comparing renography with the microsphere method of measuring intrarenal plasma flow distribution in 12 rabbits and two pigs with a variety of anaesthetics. A close agreement was found between both methods for the percentage of plasma flow distributed to the outer cortical nephrons, thus supporting the use of 'transit renography' to determine the intrarenal distribution of plasma flow.

Key words: Intrarenal haemodynamics, microspheres, renography.

Introduction

The kidney of most mammalian species, including man, consists of two nephron populations. Those with glomeruli in the outer cortex make up the majority and have relatively short loops of Henle extending only to the outer medulla, whereas the juxtamedullary nephrons have longer loops that extend to the renal papilla. Important functional differences may also exist (Jamison, 1973), thus the juxtamedullary nephrons are likely to be particularly important in conserving water whilst renin secretion is almost exclusively confined to the afferent arterioles of those in the outer cortex. Whether one particular population of nephrons has a greater capacity for sodium reabsorption is uncertain (Stein, Boonjarern, Wilson & Ferris, 1973). In view of these differences a redistribution of blood or plasma flow from one group of nephrons to the other might have a major effect on overall kidney function.

A number of techniques have been used to determine the intrarenal distribution of blood or plasma flow. In laboratory animals radioactively labelled carbon microspheres have been widely used. These are injected into the arterial circulation and, if a suitable size is used, are trapped in one circulation by the glomeruli. If the kidney is then removed and suitably sectioned, the intrarenal distribution of plasma flow can be determined from the radioactivity present. Techniques suggested for use in man include p-aminohippurate extraction,
inert gas washout, and albumin transit time. Unfortunately, the validity of all of these has been questioned (Stein et al., 1973; Nissen, 1968; Britton, Brown & Bluhm, 1971; Aukland, 1975). Another technique proposed by Britton & Brown (1971), which could have considerable clinical application, measures the transit time of 131I-labelled sodium o-iodohippurate (Hippuran) through the kidney from the renal uptake and removal functions of Hippuran, these values being obtained from an isotope renogram by deconvolution. In man, the frequency distribution of transit times has been shown to be bimodal, the peak of the first transit mode occurring at 2½–4 min with the delayed transit mode at 5–6 min (Britton & Brown, 1971; Brown & Britton, 1972). This is in accord with values of 2–4 min for the shortest transit time of inulin and p-aminohippurate, and mean transit time for inulin of 4–6 min found by others from the delay time between the injection of tracer and its appearance in the urine (Childs, Wheeler, Cominsky, Leifer, Wade & Bradley, 1955; Bradley & Wheeler, 1958). Hippuran is excreted by both glomerular filtration and tubular secretion, as is the related substance p-aminohippurate (Smith, Finklestein, Aliminosa, Crawford & Graber, 1945; Chasis, Redish, Golding, Ranges & Smith, 1945). The amount of Hippuran entering a nephron will therefore be a function of the plasma flow to the glomerulus and the environment of the proximal tubule, as radioautography has shown that the site of uptake of p-aminohippurate is confined to the proximal tubules (Wedeen & Weiner, 1973). It seems likely that the short transit mode represents the transit of the fraction of Hippuran flowing in the tubular fluid of the relatively short outer cortical nephrons and the delayed one the transit through the longer juxtamedullary nephrons. Thus the area of each transit mode might represent the proportion of plasma flowing to the respective population of nephrons.

We have tested this hypothesis by comparison with the microsphere method in rabbits and pigs. Although the two techniques may measure slightly different aspects of renal plasma flow, renography giving values representative of plasma flow to the glomeruli and proximal tubules of each nephron group whereas the microspheres determine the plasma distribution to the glomeruli alone, agreement between both methods would nevertheless provide support for the above interpretation of the renography analysis. In these studies different anaesthetic agents were used to take advantage of their profoundly different effects on the intrarenal distribution of plasma flow (Warren & Ledingham, 1975a).

Methods

Animals

Rabbits. Twelve adult New Zealand white male rabbits (weights 2·0–4·6 kg) were used. At least 3 weeks before the study a chronic indwelling polyethylene left atrial catheter was inserted by using the technique of Warren & Ledingham (1972). The catheter was filled with heparinized saline (5000 units/ml) and the free end heat-sealed. For this procedure the rabbits had been anaesthetized with intravenous pentobarbitone as necessary and intubated ‘blindly’; respiration was performed manually by using a rubber bag.

Before investigation the rabbit had been allowed food and water ad libitum. Further hydration was achieved by a 10 min infusion of 50–100 ml of 5% (0·28 mol/l) glucose into a lateral ear vein. Anaesthetic was subsequently given, also by slow injection into the ear vein, and included urethane [three rabbits, 1 g (11·2 mmol)/kg body weight], chloralose and urethane [three rabbits, chloralose 110 mg (0·35 mmol)/kg body weight; urethane 0·75 g (8·4 mmol)/kg body weight], pentobarbitone [five rabbits, 40 mg (0·161 mmol)/kg body weight], or ketamine [one rabbit, 2 mg (0·008 mmol)/kg body weight]. The rabbit was then secured in a supine position for renography and two similar collimated 1 in sodium iodide crystal scintillation detectors (J and P Engineering Ltd) were placed over the heart and left kidney, the latter being easily palpable. 131I-labelled albumin (8μCi) was injected into the ear vein and total counts during 10 s intervals recorded for 3 min. The thoracic counter and albumin injections were used to determine the ‘blood background’. Thereafter 50 μCi of 131I-labelled Hippuran was injected into the ear vein, and the total counts for 10 s intervals were recorded for 15 min. On completion of the renogram 100 000–150 000 85Sr-labelled carbonized spheres (15 μm; Minnesota Mining and Manufacturing Co.) in 0·5 ml of saline (155 mmol of NaCl/l) were injected into the left atrial catheter. Approximately 2 min later the rabbit was killed and the left kidney removed for radioactivity counting. This number of spheres has been shown to be optimal for adult rabbits (Warren & Ledingham 1975b), and a diameter of 15 μm was chosen since these are trapped by the renal glomeruli in one circulation through the kidney and are free of streaming.

**Pigs.** Similar investigations were performed on two adult female pigs, each weighing 20 kg. They were anaesthetized with Althesin (alphaxalone 0.9% (27 mmol/l), alphadolone 0.3% (7.7 mmol/l), 20 ml/h together with pancuronium as a muscle relaxant) given into the jugular vein. With the animal supine the abdomen was opened and both kidneys were exposed. For the renogram 0.5 mCi of $^{123}$I-labelled Hippuran was injected into a jugular vein. Because of the higher radioactivity of $^{123}$I as compared with $^{131}$I the renogram was recorded by a $\gamma$ camera (Nuclear Enterprises Digicamera, mark V LF) positioned to record radioactivity from both kidneys and the heart. After completing the renogram 200 000 $^{85}$Sr-labelled spheres (15 µm) were injected retrogradely into a carotid artery. The pig was later killed and both kidneys were removed for counting.

**Renography analysis.** This is fully described elsewhere (Britton & Brown, 1971; Brown & Britton, 1972) and only the principles will be outlined here. In order to determine the transit time of Hippuran through the kidney a series of curves are derived from the standard renogram (Fig. 1). The radioactivity recorded by the counter over the kidney is dependent both on that present in the kidney and that in the non-renal tissue in the field of view of the detector ('composite renogram'). If the non-renal component is eliminated ('blood background subtraction') the true kidney content curve is revealed. This is achieved by use of the other detector over the thorax and the prior injection of $^{131}$I-labelled human albumin. The ratio of radioactivity counts between the two regions is obtained. During the recording of the Hippuran renogram the Hippuran radioactivity/time curve from the detector over the thorax is scaled by using the above ratio. This scaled radioactivity/time curve is then subtracted from the composite renogram to give the true 'kidney content curve'.

The relationship between the cumulated uptake of Hippuran by the kidney and time is known as the 'uptake component'. Before Hippuran starts to leave the kidney the cumulated uptake curve is the same shape as the kidney content curve. This early part of the kidney content curve is the same shape as the integral of the plasma disappearance curve as the rate of kidney uptake is proportional to the plasma Hippuran concentration. The curve from the thoracic counter was assumed to be the same shape as the plasma disappearance curve. The relationship between the early kidney content curve and integral of the thoracic curve is derived from simple regression. The whole of the uptake component is then given by the thoracic counter integral curve multiplied by this regression coefficient. For rabbits the regression analysis used the kidney content curve between 40 and 80 s. Before 40 s the derived kidney content curve is not accurate owing to inadequate mixing. It is assumed that Hippuran starts to leave the kidney only after 80 s, as the estimated minimum transit time for $p$-aminohippurate is 100 s in the dog (Morales, Crowder, Fishman, Maxwell & Gomes, 1950). The uptake component represents the total quantity of Hippuran which has been taken up by the kidney and the kidney content curve represents that remaining in the kidney. The difference between the uptake component and kidney content curve is known as the 'removal component', as it represents the total Hippuran removed from the kidney.

The uptake component and removal component are utilized to determine the Hippuran transit times by deconvolution. The shortest transit time is given by the time of the earliest non-zero value of the removal component. The value is expressed as a fraction of the total radioactivity of the first 10 s of the uptake component. For the second 10 s of the removal component there will also be a proportion resulting from the second input period, in addition to a more delayed component from the first input period. Thus, by subtraction, the fraction of Hippuran with transit time 10 s longer than the shortest can be calculated. This continues until the whole of the removal component has been accounted for, and can be represented as a histogram.
showing the frequency distribution of transit times, which has the same shape as the curve of urine concentration with time that would be obtained if a rapid injection of Hippuran were made directly into the renal artery.

In the pigs the cumulative counts for consecutive 20 s were utilized and for determining the uptake component the integral of the plasma disappearance curve was fitted to the kidney content curve between 80 and 140 s. The \( \gamma \) camera made it possible to eliminate recorded radioactivity from around the kidney, and the camera was placed directly over the kidneys so that blood background subtraction was unnecessary.

The renography analyses were carried out using a digital computer programme and the results are expressed as the percentage of total radioactivity in the first of the two transit modes.

**Microsphere analysis**

This was based on the method of Warren & Ledingham (1975b). After removal of the capsule the pole to pole \( (d_1) \), medial to lateral \( (d_2) \) and anterior–posterior \( (d_3) \) diameters were measured with a vernier caliper. The kidney was then incubated in 10% formalin at 37°C for 48 h, at the end of which the kidney had a firm texture facilitating subsequent sectioning. The poles of the kidney were removed and three horizontal sections made through the remaining kidney with a sharp scalpel. The mean cortical thickness \( (\text{MCT}) \) was taken as the mean of 12 separate cortical measurements, four from each slice. The medulla was cut from the sharply contrasting cortex, and the cortex sectioned into outer two-thirds and inner one-third for radioactivity counting. The outer section was considered to represent predominantly cortical nephrons, the inner section predominantly juxtamedullary nephrons. Radioactivity of the sections of cortex was determined in a well-type \( \gamma \) counter. The weights of the samples varied from 0.09 to 0.82 g for rabbits and 0.81 to 3.01 g for pigs. Total radioactivity counts in 1 min ranged from 412 to 19 608. Background radioactivity was always less than a tenth of the radioactivity of the sample.

The volumes of outer and juxtamedullary cortex were calculated from \( d_1, \) \( d_2, \) \( d_3, \) total kidney weight and mean cortical thickness. If the kidney were a perfect sphere its volume would be given by

\[
\frac{4}{3} \pi \frac{(d_1, d_2, d_3)}{8}
\]

but since this is not the case a correction factor \( (c) \) has to be introduced:

\[
c = \frac{\text{kidney wt.}}{\frac{\pi d_1 d_2 d_3}{6}} = \frac{6 \text{ (kidney wt.)}}{\pi d_1 d_2 d_3}
\]

Therefore the medullary volume is given by

\[
\frac{\pi (d_1 - \frac{2}{3} \text{ MCT}) (d_2 - \frac{2}{3} \text{ MCT}) (d_3 - \frac{2}{3} \text{ MCT}) c}{6}
\]

and the volume of the juxtamedullary cortex is found by subtraction.

Assuming the specific gravity of kidney tissue to be unity the weight of the kidney may be taken as the volume. Therefore the volume of outer cortex was the difference between kidney weight and (juxtamedullary cortex + medulla). It was unnecessary to quantify the blood flow to each population of nephrons as all that was required was a simple ratio of flow between the two. Results are therefore expressed as the percentage of total radioactivity in the outer cortical zone. They have also been expressed as the ratio of the radioactivity counts/g between the two compartments of cortex.

**Blood pressure**

For rabbits systolic blood pressure was measured using a pressure cuff on the ear and visually noting through a window when blood flow in the central artery had ceased (Grant & Rothschild, 1934). For pigs pressure was measured by a transducer in the carotid artery. Blood pressure was recorded at at least 3 min intervals throughout the study and in all cases remained constant.

**Results**

The histogram of frequency distribution of transit times was bimodal. In some instances the two peaks were clearly defined (e.g. Fig. 2a), whereas in others each peak consisted of several smaller components (e.g. Fig. 2b). In the latter cases the division between the two major components could always be clearly defined. The percentage of total Hippuran radioactivity represented by the area under the first mode ranged from 14 to 82% (Table 1). For the rabbit experiments the peak of the first mode occurred at between 110 s and 140 s and
Intrarenal distributions of plasma flow

Fig. 3), but with lower values the calculated plasma flow to the outer cortical nephrons as measured by microspheres invariably exceeded the value obtained by renography. However, the overall relationship between the two techniques was highly significant \( r = +0.850, P < 0.001 \); Fig. 3).

The intrarenal plasma flow distribution was dependent on the anaesthetic used. With urethane and Althesin there was invariably >50% of the total flow distributed to the outer cortical compartment with either method of measurement, whereas both were <60% with chloralose/urethane. In the latter rabbits, however, arterial pressure was invariably lower (<70 mmHg) than for the urethane group (>70 mmHg). Four of the five rabbits anaesthetized with pentobarbitone had >60% of the total flow distributed to the outer cortical nephrons with both techniques, whereas in the other rabbit it was <50%. The difference again seemed to be related to the arterial pressure in that it was >100 mmHg in the former four rabbits, but 60 mmHg in the other one. For the rabbit anaesthetized with ketamine, however, arterial pressure was 105 mmHg, but <60% of the total activity flow was distributed to the outer cortical compartment.

Discussion

The close overall agreement between the two techniques suggests that the intrarenal distribution of plasma flow may be determined from the frequency distribution of the transit times of Hippuran through the kidney. The results were similar even with the variable effects of pentobarbitone. In instances where each of the two transit modes consisted of minor components this presumably reflects heterogeneity within each major nephron population. Exact agreement was not always obtained, especially when the percentage flow to the outer cortical nephrons was less than 60%. However, as already pointed out, the microsphere technique is a measure of glomerular perfusion, whereas renography measures both glomerular and peritubular perfusion in the area of each transit time mode. Thus differences in filtration fraction would be expected to result in slightly different results and a dissociation between the changes in plasma flow distribution and filtration fraction has been shown after saline loading (Bruns, Alexander, Riley & Levinsky, 1974).

A number of assumptions are made in carrying out the renography analysis. Renal plasma flow, the Hippuran extraction ratio, and urine flow rate

between 140 s and 230 s for the second mode. For the pigs these times were 280 s to 400 s and 420 s to 500 s respectively.

With the microsphere technique the calculated percentage plasma flow distributed to the outer cortical compartment was between 47 and 84%. When the value was greater than 60% by either method very close agreement was found (Table 1;
TABLE 1. Comparison of microsphere and renography methods for determining the percentage of plasma flow to the outer cortical nephrons

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Anaesthetic</th>
<th>Radioactivity in outer cortical compartment (%) of total</th>
<th>Ratio of counts/g for microspheres between outer cortical and juxtamedullary compartments</th>
<th>Systolic arterial pressure (mmHg)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Renography</td>
<td>Microspheres</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1</td>
<td>Urethane</td>
<td>73</td>
<td>76</td>
<td>1.95</td>
</tr>
<tr>
<td>R2</td>
<td></td>
<td>65</td>
<td>80</td>
<td>2.84</td>
</tr>
<tr>
<td>R3</td>
<td></td>
<td>59</td>
<td>64</td>
<td>1.25</td>
</tr>
<tr>
<td>R4</td>
<td>Chloralose/urethane</td>
<td>24</td>
<td>58</td>
<td>0.90</td>
</tr>
<tr>
<td>R5</td>
<td></td>
<td>40</td>
<td>54</td>
<td>0.82</td>
</tr>
<tr>
<td>R6</td>
<td></td>
<td>43</td>
<td>54</td>
<td>0.74</td>
</tr>
<tr>
<td>R7</td>
<td>Pentobarbitone</td>
<td>82</td>
<td>80</td>
<td>2.54</td>
</tr>
<tr>
<td>R8</td>
<td></td>
<td>61</td>
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<td>1.70</td>
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<td>R9</td>
<td></td>
<td>30</td>
<td>47</td>
<td>0.61</td>
</tr>
<tr>
<td>R10</td>
<td></td>
<td>75</td>
<td>64</td>
<td>1.15</td>
</tr>
<tr>
<td>R11</td>
<td></td>
<td>76</td>
<td>84</td>
<td>3.37</td>
</tr>
<tr>
<td>R12</td>
<td>Ketamine</td>
<td>14</td>
<td>51</td>
<td>0.70</td>
</tr>
<tr>
<td>P1</td>
<td>Althesin</td>
<td>(left)</td>
<td>77</td>
<td>1.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(right)</td>
<td>68</td>
<td>0.96</td>
</tr>
<tr>
<td>P2</td>
<td>Althesin</td>
<td>(left)</td>
<td>66</td>
<td>1.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(right)</td>
<td>66</td>
<td>1.04</td>
</tr>
</tbody>
</table>

are presumed to be constant, and any slight kidney movement is ignored. It is also assumed that there is no backward or forward mixing of Hippuran in its transit along the nephrons, but this is probably not valid when the tubular flow rate is markedly reduced, for during hydropenia Hippuran can be secreted into the loop of Henle (Schnermann & Thurau, 1965). This may also apply when total renal blood flow is markedly reduced. In other rabbits with a very delayed renal uptake of Hippuran, suggesting a markedly reduced blood flow, the transit curves could not be analysed into two clearly defined peaks (unpublished observations). Prior hydration is therefore essential and the technique not applicable when renal blood flow is markedly reduced.

The blood supply of the proximal tubules, which is the part of the nephron responsible for Hippuran secretion, is not necessarily from the parent glomerulus (Beeukes, 1971). The renography technique is thus likely to measure perfusion of the glomeruli and proximal tubules of each nephron population, regardless of the source of its blood supply. The method could also determine the intrarenal distribution of glomerular filtrate if a tracer substance such as $^{131}$I-labelled iothalamate were used.

Being non-invasive, 'transit renography' is suitable for use in conscious man. Radioactivity counts from thoracic and renal detectors over consecutive 20 s intervals have been found suitable (Britton & Brown, 1971; Brown & Britton, 1972; Wilkinson, Smith, Clarke, Arroyo, Richardson, Moodie & Williams, 1977). For determining the Hippuran uptake component the integral of the plasma disappearance curve (thoracic detector) is fitted to the kidney content curve between 80 and 140 s, as complete mixing may not occur earlier. It is assumed that Hippuran starts to leave the kidney only after 140 s as injected inulin only appears in the urine after 120 s (Childs et al., 1955). In humans, a bimodal frequency distribution curve for Hippuran transit times has been demonstrated in normal subjects (Britton & Brown, 1971; Brown & Britton, 1972) and cirrhosis (Wilkinson et al., 1977). In the latter, a relative reduction in the area of the first mode without any change in total renal plasma flow, suggesting a redistribution of plasma flow from outer cortical to juxtamedullary nephrons, has been shown to be related to an increased plasma renin activity.

Warren & Ledingham (1975a) used microspheres to determine the effect of different anaesthetic agents on the intrarenal blood flow distribution in rabbits. They found that pentobarbitone did not alter this and in four of five rabbits in the present study a similar normal distribution of microspheres was also found. In the other rabbit, arterial hypotension was present. Warren & Ledingham (1975a) also found that
chloralose/urethane did not alter the intrarenal blood flow distribution, in contrast to our results. However, we used higher doses of both chloralose and urethane, and hypotension was invariably present. Perfusion pressure may be an important factor determining intrarenal blood flow distribution, as has been described by others (McNay & Abe, 1970; Rector, Stein, Bay, Osgood & Ferris, 1972).

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References


