Renal Metabolism of Rabbit Serum Interferon

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SUMMARY

Three different approaches have been used to evaluate the catabolism of rabbit serum interferon (IFN) by the kidneys. Firstly, in normal rabbits the disappearance of exogenous IFN from plasma was very rapid, whereas it was significantly slower after bilateral nephrectomy. Secondly, the IFN level in arterial blood was always higher than in renal venous blood: the mean renal extraction rate of IFN in the rabbit, with a renal plasma flow of 9 ml/min, was about 1 ml/min. Thirdly, a selective and reversible tubular damage induced by maleate before intravenous administration of IFN significantly inhibited luminal uptake of IFN and markedly increases the interferonuria. All of these results support the view that the kidneys have a preponderant role in IFN filtration, catabolism and excretion.

INTRODUCTION

The sites of catabolism of interferon have not been completely defined and it was suggested (Bocci, 1977) that the kidneys may have an important catabolic role. The reasons suggesting that the kidney may affect the rapid removal of IFN from the plasma are the following: firstly, IFNs are glycoproteins with mol. wt. of about 20000 and behave as a heterogeneous mixture of polyanions (Stewart, 1979). Secondly, the glomerular filter, while almost impermeable to proteins with a mol. wt. greater than 50000, allows a transfer of smaller macromolecules that can be related to their molecular size (Brenner et al., 1976) and charge (Rennke et al., 1975). Thirdly, there is now good evidence that proteins, filtered by the glomerulus, are absorbed by the proximal tubular cells by luminal endocytosis and broken down by lysosomal enzymes (Maunsbach, 1969; Bourdeau et al., 1972). If the amount of filtered proteins exceeds the reabsorption capacity, it will in part escape tubular reabsorption and appear in the urine. Indeed the phenomenon of overflow interferonuria was described independently by Bocci et al. (1967), by Gresser et al. (1967) and by Ho & Postic (1967). However, the involvement of the kidney was not fully appreciated at that time, and because renal physiology has taken great strides during the last decade (Strober & Waldmann, 1974; Brenner et al., 1976), we have considered it worthwhile reappraising the role of the kidney in the metabolism of IFN. This paper presents results obtained by using indirect approaches, while a study with an isolated and perfused kidney is in part completed. A preliminary communication was presented at the 2nd International Workshop on Interferons (Bocci et al., 1979).

METHODS

Preparation and assay of IFN. Pooled blood IFN was obtained from 25 rabbits, 8 h after an intravenous inoculation of $10^8$ p.f.u. of Newcastle disease virus. The serum was acidified and kept at pH 2 for 7 days at $+2$ °C and then was exhaustively dialysed and concentrated against saline. After centrifugation, the clear serum was dispensed as 1 ml amounts that were kept at $-80$ °C until used. The serum IFN contained $4 \times 10^5 \pm 2.3 \times 10^5$ reference rabbit IFN IU/ml (mean of 27 titrations).
Proteins were measured by the method of Gornall et al. (1949) using a crystallized sample of rabbit serum albumin as a standard.

Interferon was titrated in serially propagated cultures of RK13 cells by measuring inhibition of vesicular stomatitis virus using the standard plaque reduction method. All titres are given in terms of the unit assigned to the research standard for rabbit IFN (preparation G-019-902-528, obtained through the courtesy of the Research Resources Branch, National Institute of Allergy and Infectious Diseases, Bethesda, Md., U.S.A.).

**Animal experiments.** New Zealand male rabbits weighing about 2-5 kg were used throughout. Bilateral nephrectomy, ligature or section of both ureters, or ligature of the renal vessels were carried out under deep anaesthesia (Farmotal) after laparotomy. In most cases each animal was used as its own control: the anaesthetized animal kept at about 38 °C by a thermostatic pad, was laparotomized, sham-nephrectomized and, after having closed the abdomen temporarily, the animal was subjected to the first measurement of IFN clearance. A dose of IFN (7 x 10⁵ IU in 3 ml saline) was injected with 0.6 mg Evans blue and heparin (1500 IU) through the cannulated right jugular vein. At predetermined times samples of 5 ml blood were withdrawn, within a few seconds, through the cannulated left carotid artery. This technique allowed exact timing that would have been impossible by bleeding the animal through the ear vein. At the end of the experiment a volume of fresh blood, equivalent to that withdrawn, was reinfused and 60 min later, bilateral nephrectomy was performed. After having withdrawn a blood sample to measure the residual percentage of IFN and Evans blue in plasma, a second dose was injected and a new determination of the plasma volume and IFN clearance was carried out. Evans blue, in the place of ¹³¹I-labelled albumin, was used to measure the plasma volume by a previously described method (Bocci & Viti, 1966). If the plasma volume and the dose of IFN are measured with great accuracy, it is possible to estimate the 100% value at the time of injection. This is the most correct method to assess the true initial point in any system with a very high turnover of the protein under study (such as IFN). After bilateral nephrectomy, there was a significant decrease of IFN disappearance (as % of dose/min) from the plasma. Graphic representation on linear paper of the IF disappearance curves allowed a planimetric calculation of the area between the two curves in a fixed period of time. The calculation was based on the assumption that, for a short period after nephrectomy, all other parameters were unchanged.

Results are reported, whenever possible, as mean ± SE. The statistical significance of the difference was evaluated according to Student’s t-test.

**Measurement of the renal arteriovenous difference in rabbits.** Nine rabbits were studied. After deep anaesthesia and heparinization (2500 IU heparin per animal) one catheter was passed from the left carotid artery into the descending aorta, while another catheter was placed in the right jugular vein and was used for returning venous renal blood and injecting IFN. Arterial pressure was continuously monitored through a by-pass inserted in the left carotid artery catheter. After laparotomy, the left renal vein was rapidly cannulated and IFN was injected through the right jugular vein; the experiment started after 3 min, so as to ensure adequate mixing of IFN in the plasma pool. IFN was administered thereafter in several doses in order to have an approximately constant plasma level throughout the experiment. At predetermined intervals simultaneous samples were collected during a period of 1 min from the aorta (via left carotid artery) and from the left renal vein (as well as from the cava, at the end of the experiment) for IFN determinations. The renal plasma flow/min was determined by measuring the venous renal blood flow/min and the haematocrit. Because of the complex operative procedure, animals underwent hypotension that could be controlled in a few experiments by slow infusion of fresh blood and nor-epinephrine.

**Inhibition of tubular interferon uptake by administration of sodium maleate.** Five rabbits, fasted for 24 h, received 140 mg maleate/kg body wt., dissolved in a vol. of 10 ml saline.
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containing 5 mM-glucose and administered half intravenously and half subcutaneously at the same time. Five control rabbits were injected with the same volume of saline–glucose. This schedule of administration is optimal (Pacini et al., 1979) in depressing significantly tubular activity without impairing the glomerular filtration rate, estimated by measuring the creatinine clearance according to Harvey & Malvin (1965).

Six h after maleate treatment, after having emptied the bladder with a catheter, rabbit serum IFN (1 × 10⁶ IU in 5 ml saline) was injected into each animal intravenously and urine was collected in ice for the following 2 h. This interval of time was selected on the basis of previous results (Pacini et al., 1979) indicating that, in the rabbit, maximal glycosuria and proteinuria occur 6 to 8 h after maleate treatment. Glucose (determined in plasma and urine by the standard glucose–oxidase method) was used as an ideal marker to assess the inhibition of reabsorption.

RESULTS

Disappearance rate of exogenous rabbit serum interferon in sham-operated rabbits and in the same animals after bilateral nephrectomy or renal vascular exclusion

A bi-exponential plasma disappearance curve was observed (Fig. 1), which could be resolved into fast and slow components. In five sham-operated animals the T/2 of the fast and slow components were 2 min ± 31 s and 18 min ± 4 min respectively, against 3 min ± 46 s and 105 min ± 7 min respectively, in the same animals after nephrectomy. The difference is highly significant (P < 0.001) for the slow component. While the fast component is mainly due to IFN dilution in the plasma pool, the slow component is more influenced by the presence or absence of the renal filter and therefore the slower disappearance rate seems to be due to the nephrectomy. Planimetric calculation of the area between the plasma IFN decay curves obtained from the same animal (used at first as the control and then after nephrectomy) allowed us to estimate that a 2.2 ± 0.8%/min decrease of IFN clearance from plasma occurred after nephrectomy.

To make sure that the disappearance curve measured in the same animal about 90 min after the first time was not due to saturation of the catabolic sites, the following experiment was carried out in one rabbit. Three doses (7 × 10⁶ IU) of serum interferon were injected: two in the sham-operated animal at an interval of 90 min and the third after nephrectomy. While the T/2 of the fast and slow components of the first clearance curve were 160 s and 19 min respectively, the T/2 of the fast and slow components of the second clearance curve were 168 s and 24 min respectively. This indicated that, in presence of a normal renal function, the disappearance rate was only slightly prolonged, most probably because, before the second injection the plasma still contained 11% of the first dose. Just before the nephrectomy the plasma contained a background of 19% of the previous dose of IFN. Following the removal of the kidneys and injection of the third dose of IFN the T/2 of the fast and slow components was raised to 230 s and 140 min, indicating that, without kidneys, there is indeed a marked delay in the elimination of IFN from plasma.

Moreover the disappearance rate of IFN has been measured after the ligature of both ureters, the same two animals being used as control. The T/2 of the fast and slow components averaged 165 s and 21 min respectively, in the sham-operated rabbits, and increased to 171 s and 53 min respectively, after ureter ligature suggesting that blocking the urinary flow slowed the disappearance rate from plasma. A longer T/2 for the slow components would probably be found with chronically ligated (24 h before) ureters but we have not used this condition as uraemic toxins could alter the normal catabolic pathways. In contrast, rabbits which had undergone section of the ureters cleared plasma IFN as well as the controls indicating that in
Fig. 1. Disappearance rate of exogenous rabbit serum interferon as a function of time in five sham-operated rabbits (●) and in the same animals injected with interferon after bilateral nephrectomy (○). Each point is the mean ± SE.

Fig. 2. Disappearance rate of exogenous rabbit serum interferon as a function of time in two controls (●, ■) and in the same rabbits injected with interferon after ligature of renal vessels (○, □). Each point is the mean ± SE.

the absence of increased intratubular pressure (presumably present after ureter ligature) IFN filtration is unimpeaded.

Finally, disappearance of IFN from plasma is also considerably delayed after ligature of the renal vessels. Fig. 2 shows clearance curves measured in two rabbits before and after ligature of the renal pedicles. In this case the control clearance study was performed on the anaesthetized, but otherwise intact animal, followed, after 1 week, by the second clearance study after the operation.

Renal extraction of interferon

The results of only two experiments are presented in Table 1 because several others had to be discarded owing to irreducible arterial hypotension. It may be significant to note that if arterial pressure was below 60 mm Hg no extraction of IFN could be detected with the current assay method for IFN. Although it has not been possible to collect enough urine from the left kidney (the vein of which was cannulated) to measure the creatinine and interferon clearances, it was observed that: (i) the concentration of IFN in the renal vein was always lower than in the artery irrespective of the high and low IFN levels in plasma; (ii) the mean IFN extraction by the kidney was 11% with a range from 9 to 16%. Therefore, with a renal plasma flow of about 9 ml/min, the mean renal extraction rate of interferon is about 1 ml of plasma/min for each kidney. This means that in a rabbit of 3 kg the kidneys alone could eliminate the IFN from the plasma pool within 63 min.

Impairment of tubular IFN absorption and interferonuria after maleate administration

Judicious administration of maleate in some experimental animals can selectively and reversibly impair tubular absorption of several small mol. wt. solutes (Berliner et al., 1950; Harrison & Harrison, 1954) and of filtered proteins with mol. wt. below 50000 (Mogielnicki et al., 1971). Table 2 shows that, after administration of maleate (140 mg/kg body wt.), the
<table>
<thead>
<tr>
<th>Expt. no.</th>
<th>Time intervals (min)</th>
<th>Arterial pressure (mm Hg)</th>
<th>Carotid artery (CA) (IFN units/ml)</th>
<th>Renal vein (RV) (IFN units/ml)</th>
<th>RV extraction ratio ( \times 100 )</th>
<th>IFN extraction ratio ( \frac{CA}{CA - RV} )</th>
<th>RV flow (ml/min)</th>
<th>Arterial haematocrit (%)</th>
<th>RV plasma flow (ml/min)</th>
<th>Renal extraction rate of IFN (ml/min)</th>
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</thead>
<tbody>
<tr>
<td>6</td>
<td>3–4</td>
<td>85</td>
<td>4100</td>
<td>3700</td>
<td>90.2</td>
<td>0.098</td>
<td>12</td>
<td>34</td>
<td>7.9</td>
<td>0.77</td>
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<td></td>
<td>7–8</td>
<td>100</td>
<td>4000</td>
<td>3350</td>
<td>83.8</td>
<td>0.162</td>
<td>11.5</td>
<td>33</td>
<td>7.7</td>
<td>1.25</td>
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<tr>
<td></td>
<td>11–12</td>
<td>100</td>
<td>4400</td>
<td>3800</td>
<td>86.4</td>
<td>0.136</td>
<td>13.5</td>
<td>32</td>
<td>9.2</td>
<td>1.25</td>
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<td></td>
<td>15–16</td>
<td>95</td>
<td>4600</td>
<td>4100</td>
<td>89</td>
<td>0.11</td>
<td>12.5</td>
<td>33</td>
<td>8.4</td>
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<tr>
<td>Mean ± se</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>87.3 ± 2.8</td>
<td>0.126 ± 0.03</td>
<td>8.3 ± 0.67</td>
<td>1.05 ± 0.24</td>
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<tr>
<td>9</td>
<td>3–4</td>
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<td>16000</td>
<td>14500</td>
<td>90.6</td>
<td>0.09</td>
<td>16</td>
<td>34</td>
<td>10.6</td>
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<tr>
<td></td>
<td>11–12</td>
<td>80</td>
<td>17000</td>
<td>15000</td>
<td>88.2</td>
<td>0.117</td>
<td>16</td>
<td>33</td>
<td>10.7</td>
<td>1.25</td>
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<tr>
<td></td>
<td>15–16</td>
<td>85</td>
<td>18500</td>
<td>16500</td>
<td>89.1</td>
<td>0.108</td>
<td>15</td>
<td>33</td>
<td>10.1</td>
<td>1.09</td>
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<td></td>
<td>20–21</td>
<td>75</td>
<td>21300</td>
<td>19200</td>
<td>90.1</td>
<td>0.098</td>
<td>13</td>
<td>32</td>
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<tr>
<td>Mean ± se</td>
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<td></td>
<td></td>
<td></td>
<td>89.5 ± 1.1</td>
<td>0.103 ± 0.01</td>
<td>10.1 ± 0.9</td>
<td>1.04 ± 0.17</td>
<td></td>
<td></td>
</tr>
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</table>

Table 1. Renal extraction of homologous serum IFN in rabbits as estimated by determination of the IFN level in arterial and renal venous blood samples.
Table 2. Recovery of IFN from rabbit urine after intravenous administration of $1 \times 10^6$ IU of homologous serum interferon* in normal and maleate-treated rabbits

<table>
<thead>
<tr>
<th>Samples</th>
<th>Creatinine clearance (ml/min)</th>
<th>Total glycosuria (mg)</th>
<th>% Glucose fractional reabsorption</th>
<th>Interferonuria (% of dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.8 ± 1.9</td>
<td>0</td>
<td>100</td>
<td>6.8 ± 2.5</td>
</tr>
<tr>
<td>Maleate</td>
<td>8.5 ± 1.6</td>
<td>400 ± 195</td>
<td>67</td>
<td>23.7 ± 7.1</td>
</tr>
</tbody>
</table>

* IFN was injected 6 h after maleate administration and urine was collected for the following 2 h.

glomerular filtration rate (as measured with creatinine) was very similar to that of the controls, while the tubular reabsorption of glucose was markedly impaired. In fact a considerable amount of glucose (absent in the controls) was measured in the urine of the maleate-treated rabbits. Similarly, interferonuria was significantly ($P < 0.01$) raised in the maleate-treated animals indicating reduced tubular uptake of IFN.

**DISCUSSION**

In the last decade a great deal of evidence (Bernier & Conrad, 1969; Mogielnicki *et al.*, 1971; Peterson & Berggard, 1971; Katz & Rubenstein, 1973; Kau & Maack, 1977; Johnson & Maack, 1977; Naets & Wittek, 1974), has accumulated showing that the kidneys play a decisive role in the turnover of proteins having mol. wt. below 50000. Thus, it has become worthwhile to reappraise the role of the kidney in the catabolism of IFN.

In the present experiments the problem has been studied with three different indirect approaches. Both the comparative determinations of the disappearance rate of IFN in control and nephrectomized rabbits and the measurement of the renal extraction rate of IFN strongly suggest that the kidneys are involved in the rapid removal of IFN from the circulation. The maleate-induced interferonuria in experimental animals is a very useful model for studying possible impairment of the tubular uptake of proteins and closely reproduces the symptoms observed in the Fanconi syndrome (Mogielnicki *et al.*, 1971). We have now shown that appropriate administration of maleate does not damage glomerular filtration but markedly inhibits luminal reabsorption of glucose and IFN. After intravenous administration of IFN, the amount present in the plasma and hence in the filtrate may exceed the absorption capacity of the tubular cells even in normal conditions as it has been found in the rabbit (Bocci *et al.*, 1967; Ho & Postic, 1967), mouse (Gresser *et al.*, 1967) and man (Emodi *et al.*, 1975; Arvin *et al.*, 1976). This situation can be defined as overflow interferonuria and it may be useful to emphasize that intravenous administration of large amounts of IFN in humans for therapeutic purposes may lead, at least transiently, to considerable and wasteful loss of IFN in the urine. Moreover, if the tubular absorptive function is impaired, the recovery in the urine of the injected IFN may considerably increase and this situation can be defined as tubular interferonuria. This aspect has not yet been appreciated in man but it should be kept in mind particularly now that clinical trials are including more patients, some of which may have interstitial nephritis.

Using a direct approach, such as studying the filtration, absorption and excretion of human IFN-α with an isolated and perfused rabbit kidney, Bocci *et al.* (1981) have estimated a fractional turnover rate of 0.85% per min per kidney, a value that is in good agreement with the renal extraction rate measured in the present experiments.

Thus the catabolic role of the kidney is emerging: IFN molecules present in the glomerular plasma leak fairly easily into the filtrate (Brenner *et al.*, 1976; Bocci, 1981). Once IFN molecules have leaked into the tubular fluid, they will move along the proximal tubule and are more or less completely absorbed by segregation into endocytotic vesicles at the apical
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borders of the tubular cells. This pathway is well proved (Christensen & Maunsbach, 1974; Maack et al., 1979; Carone & Peterson, 1980) for many other plasma proteins. Obviously if the concentration of IFN in the tubular fluid is high enough, it can saturate the uptake mechanism and interferonuria will ensue. But because the absorption capacity is normally very efficient the interferonuria will be almost negligible and it was this finding (Bocci et al., 1967; Ho & Postic, 1967) that lead to the incorrect conclusion that the catabolic role of the kidney was of little significance. In fact the kidney and specifically the tubular cells appear now to have a major catabolic role. As labelled IFN is now becoming available, it is hoped to be able to visualize the intracellular catabolism in the near future.

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