
ESTIMATION OF RENAL BLOOD FLOW BY USE OF ENDOGENOUS N1-METHYLNICOTINAMIDE IN RATS*

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The usefulness of the renal clearance of endogenous N1-methyl nicotinamide (NMN) as a probe for estimating the renal blood flow (RBF) was examined in rats. And the effect of experimental acute renal failure (ERF) on RBF was also examined in rats treated with glycerol, folate, salicylate, uranium and gentamicin by using this method. No significant difference was shown between the values of RBF determined by the NMN method and conventional p-aminohippurate (PAH) method in both the intact (control) and glycerol-ERF rats, suggesting the usefulness of the NMN method in determining RBF. No significant difference was also shown in RBF between the control and all ERF-rats studied, through significant decreases in the renal clearance of NMN and renal extraction ratio (ER) were observed in the ERF-rats except the gentamicin-treated rats. It was suggested that the endogenous NMN would be a useful probe to estimate RBF without constant infusion of exogenous substances.

Keywords — renal blood flow; endogenous N1-methyl nicotinamide; experimental acute renal failure; glycerol; folate; salicylate; uranium; gentamicin

INTRODUCTION

It is important to estimate the renal blood flow (RBF) in assessing the kidney function in patients with renal disfunction. To estimate RBF, p-aminohippurate (PAH) clearance has been widely used.11 This method, however, has some shortcomings; e.g. 1) a constant-infusion is required to attain a steady-state plasma concentration of PAH and this may give a physiological burden to the patients, 2) PAH, an organic anion, may affect the kidney function such as the tubular secretion of organic anionic drugs, and 3) the extraction ratio (ER) of PAH by the kidney may be changed in diseases as reported in the dog with experimental azotemia.21

Endogenous N1-methyl nicotinamide (NMN), an organic cation, is biosynthesized from nicotinamide in the mammalian liver and is excreted in the urine both by the glomerular filtration and the proximal tubular secretion, with negligible tubular reabsorption.21

In the present study, we examined the usefulness of the renal clearance of endogenous NMN as a probe for estimating RBF in the rat with experimental renal failures (ERF). The ERF-rats were produced by the reported methods with glycerol,4, 5 folate,6-8 salicylate,9 uranium,10,11 and gentamicin.12-14 Inulin clearance was used as the glomerular filtration rate (GFR).

MATERIALS AND METHODS

Materials — NMN and folic acid were purchased from Tokyo Kasei Co., Tokyo, Japan. Inulin, mannitol and glycerol were purchased from Wako Pure Chemicals Ind. Ltd., Tokyo, Japan. PAH was purchased as 20 v/v % solution

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from Daiichi Pure Chemicals Co., Tokyo, Japan. Sodium salicylate was purchased from Koso Chemicals Co., Tokyo, Japan. Gentamicin sulfate (570 \( \mu \)g/mg) was obtained from Shionogi Pharmaceutical Co., Osaka, Japan. \( ^{3}H \)-inulin (257.6 mCi/g) were purchased from New England Nuclear Co., Boston, MA. All other chemicals were reagent grade and used without further purification.

**Animals** — Male Wistar rats weighing 210—280 g were used in all experiments. Water and commercial chow (CE-2, Clea Japan Inc., Tokyo) were given ad libitum. Glycerol renal failure was produced by injection of 10 ml/kg of glycerol solution (50 v/v % in saline) subcutaneously at 24 h prior to the clearance experiment. Folate renal failure was produced by injection of 5 ml/kg of folic acid (10.5 w/v % in 0.3 M sodium bicarbonate) intravenously at 24 h prior to the experiment. Salicylate renal failure was produced by injection of 2.5 ml/kg of sodium salicylate solution (160 v/v % in saline) intravenously at 48 h prior to the experiment. Gentamicin—renal failure was produced by injection of 5 ml/kg of gentamicin sulfate solution (0.8 w/v % in saline; stored at 4 °C) subcutaneously at 24 h interval for 10 d. The clearance experiment was performed at 24 h after the final (10-th) injection. Normal rats without any treatment were used as the control.

**Experimental Procedure** — Under light ether anesthesia, the femoral vein and artery were cannulated with polyethylene tubings (PE-50; Clay Adams, Becton Dickinson & Co., Parsippany, N.J.) for the drug administration and blood sampling, respectively. After the abdomen was opened through a midline incision, both ureters were cannulated with PE-10 (polyethylene tubings). The tip of an injection needle (25 gauge) attached to a PE-50 (polyethylene tubing) was inserted into the renal vein of left kidney and was fixed with surgical glue (Aron Alpha, Sankyo Co. Ltd., Tokyo, Japan). The needle was bent in some cases for the convenience of insertion. This cannula was connected to a 1-ml syringe and a small volume of renal venous blood was drained by pulling the syringe very gently. After confirmation of the blood draining, the syringe was changed to another syringe filled with saline and the blood in the cannula was pushed back up to the cannulated point by saline (in the syringe) and then the abdomen was sutured. The clearance study was started at more than 1 h after the operation to recover from ether anesthesia. The body temperature was maintained at 37 °C with a heat lamp. Mannitol solution (3 w/v % in saline) was infused at a constant rate of 3.0 ml/h into a femoral vein via a PE-50 cannula throughout the experiment. For the estimation of the renal plasma flow (RPF) by the PAH method, PAH was infused at a constant rate of 30 mg/h/rat a loading dose of 100 mg/kg. Blood samples (0.12 ml) were taken at 0, 20 and 40 min from both femoral artery and renal vein via a PE-50 cannula into heparinized polyethylene centrifuge tubes and were centrifuged at 3000 rpm for 4 min in a table top centrifuge (Beckman Instrument, Fleton, CA). Urine samples were collected at the same sampling intervals as those of blood. Plasma and urine samples were stored at -20 °C until assay for NMN or PAH.

The clearance of NMN or PAH was calculated by \( CL_{\text{P}} = (C_{u} \times V_{u}) / (C_{a} \times t) \), where \( CL_{\text{P}} \) is the renal clearance (ml/min/kg), \( C_{u} \) is the concentration in urine (\( \mu \)g/ml), \( C_{a} \) is the mean plasma concentration during urine-collecting period (\( \mu \)g/ml), \( V_{u} \) is the urine volume normalized for the body weight (ml/kg), and \( t \) is the urine collecting time (min), respectively. The extraction ratio (ER) of NMN or PAH by the kidney was calculated by \( ER = (C_{a} - C_{v}) / C_{a} \), where \( C_{a} \) and \( C_{v} \) are the arterial and renal venous plasma concentrations of NMN or PAH, respectively.

The distribution ratio of NMN from plasma to red blood cells (\( R_{b} \)) was determined as follows. Two hundreds \( \mu \)l of arterial blood was placed in a heparinized test tube and was added.
10 μl of 0.2 w/v % NMN solution. After incubation at 37 °C for 10 min with stirring, the blood was centrifuged at 3000 rpm for 1 min to obtain the plasma sample. The hematocrit value (Hₜ) of the incubated blood sample was determined using the hematocrit tube (VC-HO 75H, Terumo, Tokyo, Japan) after centrifugation at 3000 rpm for 4 min.

Since the Rₜ of NMN was negligible, RPF can be calculated by the conventional equation

$$\text{RPF} = \frac{CL_{P}}{ER}$$

and RBF was also calculated by

$$\text{RBF} = \frac{\text{RPF}}{1 - H_{t}}$$

**Assay Procedure** — The concentration of NMN in plasma was assayed by the modified method of Clark et al. Briefly, 0.3 ml of 17 w/v % trichloroacetic acid (TCA) was added to 50 μl of plasma sample diluted properly with distilled water and was stirred. After centrifugation, the supernatant was decanted and washed twice with 3 ml of water-saturated ethylacetate. Two hundreds μl of the aqueous layer was transferred into a test tube, and then ethyl alcohol (0.8 ml), acetophenone (0.1 ml) and 3 M KOH (0.1 ml) in 80 v/v % ethyl alcohol were added successively and well stirred. Exactly 8 min after the addition of 3 M KOH, 3.0 ml of formic acid was added and stirred immediately. The reagents were added to 0.2 ml of water in the tube serving as the blank. The fluorescence was measured at 430 nm (excited at 370 nm) in a Hitachi MPF-4 spectrofluorometer (Hitachi Ltd., Tokyo, Japan) within 48 h after the addition of formic acid. A standard curve was prepared by adding acetophenone, 3 M KOH and formic acid as described above to the tube containing different concentrations (0.04—0.16 μg/ml) of NMN in 1 ml of 80 v/v % ethyl alcohol. Over this concentration range, the intensities were linearly related to the concentration of NMN with a correlation coefficient of at least 0.980. The limitation of the sensitivity of the assay was 20 μg/ml. The TCA extract from serum containing exogenous authentic NMN, yielded more than 89% of the fluorescence intensity of similarly treated TCA extracts from 10⁻⁴ M HCl containing the same amount of NMN. For the urine, a 0.2 ml aliquot of the diluted urine samples with distilled water (40 times or more) was used for the determination of NMN as described above. The concentration of endogenous NMN was also determined using the blank plasma or urine sample. PAH was determined by the method of Bratton and Marshall.

**Statistical Analysis** — The regression lines of the two groups of data were obtained by the linear least squares method. The difference be-

### TABLE I. Renal Plasma Flow (RPF) and Renal Blood Flow (RBF) Determined by Endogenous NMN in the Control and ERF-Rats

<table>
<thead>
<tr>
<th></th>
<th>Control (n=6)</th>
<th>Glycerol (n=6)</th>
<th>Folate (n=3)</th>
<th>Uranium (n=3)</th>
<th>Salicylate (n=4)</th>
<th>Gentamicin (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPF, ml·min⁻¹·kg⁻¹</td>
<td>30.02 ± 3.95</td>
<td>25.31 ± 4.82</td>
<td>34.08 ± 7.69</td>
<td>24.95 ± 3.35</td>
<td>24.02 ± 6.04</td>
<td>26.76 ± 3.56</td>
</tr>
<tr>
<td>RBF, ml·min⁻¹·kg⁻¹</td>
<td>55.49 ± 7.82</td>
<td>38.94 ± 7.42</td>
<td>58.46 ± 13.19</td>
<td>47.60 ± 6.38</td>
<td>42.74 ± 10.75</td>
<td>49.28 ± 6.56</td>
</tr>
<tr>
<td>CLₚ, ml·min⁻¹·kg⁻¹</td>
<td>21.75 ± 3.37</td>
<td>7.22 ± 1.13</td>
<td>8.20 ± 2.00</td>
<td>1.36 ± 1.73</td>
<td>12.06 ± 2.49</td>
<td>16.85 ± 3.71</td>
</tr>
<tr>
<td>ER (b)</td>
<td>0.76 ± 0.03</td>
<td>0.31 ± 0.06</td>
<td>0.25 ± 0.07</td>
<td>0.06 ± 0.03</td>
<td>0.53 ± 0.05</td>
<td>0.65 ± 0.14</td>
</tr>
<tr>
<td>Hₜ (b)</td>
<td>0.459 ± 0.005</td>
<td>0.350 ± 0.011</td>
<td>0.417 ± 0.007</td>
<td>0.476 ± 0.023</td>
<td>0.438 ± 0.008</td>
<td>0.457 ± 0.003</td>
</tr>
</tbody>
</table>

(a) Each value represents the mean ± s.e. for each group. (b) significantly different from the control rats at p=0.001, (c) significantly different from the control rats at p=0.05, (d) extraction ratio by the kidney (see Text), (e) significantly different from the control rats at p=0.01, (f) hematocrit value.
TABLE II. Renal Plasma Flow (RPF) Determined by the PAH Method a)  

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 3)</th>
<th>Glycerol (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPF, ml min⁻¹ kg⁻¹</td>
<td>29.01 ± 3.25</td>
<td>33.41 ± 5.17</td>
</tr>
<tr>
<td>CLṛ, ml min⁻¹ kg⁻¹</td>
<td>28.73 ± 3.12</td>
<td>14.60 ± 3.88 b)</td>
</tr>
<tr>
<td>ER c</td>
<td>0.99 ± 0.001</td>
<td>0.42 ± 0.04 b)</td>
</tr>
</tbody>
</table>

a) Each value represents the mean ± s.e. for each group, b) significantly different from the control rats at p = 0.05, c) extraction ratio by the kidney (see Text).

![Correlation between the Extraction Ratio (ER) of NMN by the Kidney and the Renal Plasma Clearance (CLṛ) of NMN in the Control and ERF-Rats](image)

FIG. 1. Correlation between the Extraction Ratio (ER) of NMN by the Kidney and the Renal Plasma Clearance (CLṛ) of NMN in the Control and ERF-Rats

\[ y = 26.75x - 0.29 \quad (n = 25), \quad r = 0.843 \quad (p < 0.001) \]

Key: (●) control; (○) glycerol-; (□) folate-; (△) uranium-; (■) salicylate-; and (▲) gentamicin-ERF rats.

The results of RPF and RBF determined by the NMN method are listed in Table I, with those of CLṛ, ER and Hₑ. No significant difference was shown in both RPF and RBF between the control and all ERF-rats, though significant decreases in CLṛ and ER were observed in the ERF-rats except the gentamicin-ERF rats. Also significant decreases in Hₑ were observed in the glycerol- and folate-ERF rats.

In the gentamicin-ERF rats, though GFR greatly decreased, a slight decrease in CLṛ was observed. This finding suggests a glomerulo-tubular imbalance.

Table II shows the results of RPF determined by the conventional PAH method in the control and glycerol-ERF rats with those of CLṛ and ER. Significant decrease in CLṛ and ER were observed in the glycerol-ERF rats. No significant difference was observed in the values of RBF determined by the PAH method as compared to those determined by the NMN method (Table I) in both the control and glycerol-ERF rats, suggesting the usefulness of the NMN method to estimate RBF. The values of RPF in normal rats (Table I) were comparable with those reported by Dedrick et al.19

Figure 1 shows the relationship between ER and CLṛ of NMN in the control and ERF-rats, and a significant (p < 0.001) correlation was ob-
served between the two groups. This suggests that the decrease in the renal clearance of NMN in the ERF-rats may not be due to the decrease in RPF\textsuperscript{20,21} but to the decrease in the renal tubular secretory function.\textsuperscript{22} In fact the renal extraction ratio of PAH was nearly equal to 1 (Table II) in the control rats and it is not necessary to sample the renal venous blood to determine RBF. In the renal failure, however, the decrease in ER of PAH is observed as shown in the glycerol-ERF rats (Table II) and therefore it is necessary to determine the concentration of PAH in the renal venous blood for the calculation of ER.

In conclusion, it was suggested that the endogenous NMN would be a useful probe to estimate RBF without constant infusion of exogenous substances.

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**REFERENCES**


