Note

Course of the Urinary Excretion of Nicotinic Acid and Its Related Compounds Following the Injection of Optimal Amount of Nicotinic Acid for NAD Synthesis

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When a physiological amount of nicotinic acid (NiA) (about 5 mg/kg body weight) is administered to rats, it is efficiently synthesized into NAD as well as nicotinamide (Nam)¹⁾ and the pattern of urinary excretory metabolites such as *N*¹-methylnicotinamide (MNA), *N*¹-methyl-2-pyridone-5-carboxamide (2-py), and *N*¹-methyl-4-pyri-

done-3-carboxamide (4-py) is basically the same as that of Nam.¹⁾ However, when a large amount of NiA (about 500 mg/kg body weight) is administered to rats, little of the NiA is metabolized into Nam, and its direct metabolites such as MNA, 2-py, and 4-py,¹⁾ because high concentration of NiA inhibits NAD synthesis *in vivo* from either Nam or NiA.^{2,3)} It is reported that the optimal concentration of NiA for NAD synthesis (the concentration that the maximum NAD level in liver is attained) is 50~150 mg/kg body weight in rats.¹⁾ In this amount of NiA, it is not reported what pattern of urinary excretion of NiA and its related compounds is obtained. In this experiment, we investigated the pattern of urinary excretion of NiA and its related compounds when the optimal concentration of NiA for NAD synthesis is administered to rats.

The contents of Nam, 2-py, and 4-py in urine were simultaneously measured by the high-performance liquid chromatographic (HPLC) method of Shibata *et al.*⁴⁾ and the contents of NiA and nicotinuric acid (NuA) were simultaneously measured by the HPLC method of Shibata.⁵⁾ The content of MNA in urine was measured by the HPLC method of Shibata⁶⁾ and the content of creatinine was measured by the HPLC method of Shibata.⁷⁾

Table I shows the course of the urinary excretion of NiA

Table I. COURSE OF THE URINARY EXCRETION OF NiA AND ITS METABOLITES FOLLOWING THE NiA INJECTION

Male rats of the Sprague-Dawley strain (seven weeks old) were purchased from Clea Japan Inc. The rats were kept individually in metabolic cages, with a 06:00~18:00 (light) and 18:00~06:00 (dark) schedule. The room temperature was kept at 22±2°C and the humidity was about 60%. A 20% casein diet^a and water were fed *ad libitum* to the rats. After being kept for 27 days (rat body weight 350.3±36.4 g), 25 mg (203 μmol) of NiA per rat (about 70 mg NiA/kg body weight), dissolved in 1 ml of sterilized saline was intraperitoneally injected at 9:00 a.m. Twenty-four hour urine from the day before the NiA injection was collected, and urine was collected for 0~5, 5~10, and 10~24 hr in a flask containing 1 ml of 1 M HCl after the injection and stored until use at -25°C.

	0~5 hr	5~10 hr	10~24 hr	0~24 hr
Before the NiA injection				
NiA (nmol/μmol creatinine)	— ^b	—	—	N.D. ^c
NuA (nmol/μmol creatinine)	—	—	—	N.D.
Nam (nmol/μmol creatinine)	—	—	—	11±4
MNA (nmol/μmol creatinine)	—	—	—	9±3
2-py (nmol/μmol creatinine)	—	—	—	10±2
4-py (nmol/μmol creatinine)	—	—	—	124±44
After the NiA injection				
NiA (nmol/μmol creatinine)	2682±417	226±164	21±15	526±78
NuA (nmol/μmol creatinine)	2770±623	565±488	36±22	629±147
Nam (nmol/μmol creatinine)	57±16	116±21	31±3	47±5
MNA (nmol/μmol creatinine)	27±11	119±47	60±51	63±40
2-py (nmol/μmol creatinine)	25±7	81±10	53±16	53±12
4-py (nmol/μmol creatinine)	272±105	584±212	250±155	313±107

^a K. Shibata R. Nomamoto and K. Iwai, *Agric. Biol. Chem.*, **52**, 1765 (1988).

^b Not measured.

^c Not detected. Values are means±S.D. for 6 rats.

Table II. INCREASE IN THE URINARY EXCRETION OF NiA AND ITS METABOLITES FOLLOWING THE NiA INJECTION

	$\mu\text{mol}/24\text{ hr}$ urine	% of dose
Increase in urinary excretion ^a of		
NiA	48.6 ± 8.4	23.9 ± 4.1
NuA	55.8 ± 10.6	27.5 ± 5.2
NiA + NuA	104.4 ± 8.4	51.4 ± 4.1
Increase in urinary excretion ^a of		
Nam	3.5 ± 0.5	1.7 ± 0.3
MNA	4.9 ± 3.1	2.4 ± 1.5
2-py	4.0 ± 1.0	2.0 ± 0.5
4-py	19.2 ± 8.6	9.5 ± 4.2
Nam + MNA + 2-py + 4-py	32.6 ± 5.5	16.1 ± 2.7

^a Calculated from (values of 24 hr urine after the NiA injection) — (values of 24 hr urine before the NiA injection).

Experimental conditions, see Table I.

and its metabolites following the NiA injection and Table II shows the increases in the urinary excretion of NiA and its metabolites for 24-hr urine after the NiA injection. The highest urinary excretion of NiA and its direct metabolite, NuA was observed 0~5 hr after the NiA injection and after that time, these excretion decreased sharply. On the other hand, the highest urinary excretion of Nam and its direct metabolites such as MNA, 2-py, and 4-py was observed 5~10 hr after the NiA injection. The increased NAD caused by the injection of NiA is hydrolyzed into Nam and then the Nam is rapidly metabolized into MNA, 2-py and 4-py. This is the reason why the excretion of

Nam, MNA, 2-py, and 4-py is delayed. When a large amount of NiA (500 mg/kg) was injected, 82.5% of the dose was excreted unchanged form, 10% of the dose as NuA for 0~24 hr urine, and only 5.6% of the dose was excreted as Nam and its direct metabolites such as MNA and 2-py.¹⁾ In that report,¹⁾ the urinary excretion of 4-py was not detected, but in this experiment, 10% of the NiA dose was metabolized into 4-py. In this experiment, about 24% of the dose was excreted unchanged, about 28% of the dose as NuA, and about 16% of the dose as Nam, MNA, 2-py, and 4-py for 0~24 hr after the NiA injection. This result means that at least about 30 μmol of NiA is metabolized into NAD [$203 \mu\text{mol}$ (injected amount of NiA) $\times 0.16 = 30 \mu\text{mol}$]. The total urinary excretion of NiA, NuA, Nam, MNA, 2-py, and 4-py was about 70% of the dose for 24 hr urine after the NiA injection. From these findings, it was found that when the optimal concentration of NiA for NAD synthesis was administered to rats, the NiA was efficiently metabolized into Nam, MNA, 2-py, and 4-py.

References

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