Note

Effects of Pyrazinamide on Tryptophan–Niacin Conversion in Rats

Katsumi SHIBATA

Department of Food Science and Nutrition, Teikoku Women's University, Moriguchi, Osaka 570, Japan

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Nasu *et al.*[1] have found that pyrazinamide loading markedly increased the urinary excretion of quinolinic acid, but that the urinary excretion of nicotinamide and N^1-methylnicotinamide (MNA) was not significantly increased. They also demonstrated that the increase in quinolinic acid excretion resulted from the inhibition of aminocarboxymuconate-semialdehyde decarboxylase (ACMSDase) by an unknown metabolite of pyrazinamide. This suggests that either the reaction quinolinic acid→nicotinic acid mononucleotide *in vivo* as well as the ACMSDase reaction is inhibited by pyrazinamide loading, or that the increase in quinolinic acid formation does not contribute to the increase in nicotinic acid mononucleotide formation because quinolinate phosphoribosyltransferase is already saturated by substrate quinolinic acid under normal conditions. To check the above possibility, I investigated the effect of pyrazinamide on the tryptophan-niacin metabolism in a longer period than Nasu's experiment.[1]

MNA chloride, kynurenic acid, and pyrazinamide were purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). N^1-Methyl-2-pyridone-5-carboxamide (2-Py) and N^1-methyl-4-pyridone-3-carboxamide (4-Py) were synthesized by the methods of Pullman and Colowick[2] and Shibata *et al.*[3] Nicotinamide was purchased from Wako Pure Chemical Industries (Osaka, Japan). All the other chemicals used were of the highest purity obtainable from commercial sources.

Rats of the Wistar strain (3 weeks old) were purchased from Clea Japan (Tokyo, Japan). The rats were immediately placed in individual metabolic cages (CT-10; Clea Japan) and fed *ad libitum* a nicotinic acid-free, 20% casein diet [vitamin-free milk casein, 20%; L-methionine, 0.2% α-cornstarch, 45.8% sucrose, 23%; corn oil, 5%; mineral mixture (Oriental's ration),[4] 5%; nicotinic acid-free vitamin mixture (Oriental's ration),[4] 1%] during the experiment. At 09:00 and 17:00 hr on day 39, the rats (body weight, 260.0±4.5 g, mean±SEM, $n=5$) were twice injected intraperitoneally with a large amount of pyrazinamide (100 mg/kg body weight) dissolved in saline (1 ml). Twenty-four-hour urine samples (09:00–09:00) from the previous day (day 38) and the next 5 days (day 39–43) were collected into flasks containing 1 ml of 1 M HCl and stored at −25°C until analyzed for kynurenic acid, nicotinamide, and its metabolites. The room temperature was kept at 22±2°C the humidity was about 60% and a 12-hr light/dark cycle was maintained.

Contents of MNA and kynurenic acid in the urine were measured by the high-performance liquid chromatographic

Table I. DAILY BODY WEIGHT GAIN AND FOOD INTAKE, AND URINARY EXCRETION OF NICOTINAMIDE AND ITS METABOLITES BEFORE AND AFTER INJECTING RATS WITH A LARGE AMOUNT OF PYRAZINAMIDE

	Day before injection	Day 1	Day 2	Day 3	Day 4	Day 5
Daily body weight gain (g/day)	4.2±0.1	4.4±0.3	4.4±0.2	4.4±0.3	4.5±0.1	4.4±0.2
Day food intake (g/day)	15.5±1.5	15.5±1.0	14.6±0.4	16.0±0.5	15.8±0.6	15.4±0.3
Nam	113±7^a	1384±82^b	447±33^c	227±11^d	174±14^e	119±18^a
MNA	366±21^a	5008±332^b	3355±283^c	804±71^d	354±27^a	427±63^a
2-Py	91±7^a	3285±226[1],^{b}	907±46^c	245±12^d	140±9^a	95±9^a
4-Py	1392±108^a	12035±91^b	7067±144^c	2746±81^d	1789±91^e	1450±120^a
Sum[2]	1962±129^a	21712±1388^b	11777±357^c	4023±118^d	2458±136^a	1847±137^a
KA[3]	1885±122	2022±112	1746±105	1974±111	1871±130	1882±124

Values are expressed as nmol/day and are means±SEM for 5 rats; values with different superscript letters in the same row are statistically significantly different at $p<0.05$ by Duncan's new multiple range test.[14]

[1] Standard pyrazinamide was eluted with 2-Py under the analytical conditions of 2-Py, and pyrazinamide is also extracted with diethyl ether, so this value might include pyrazinamide. Pyrazinamide used in this experiment was pure in these analytical conditions.[3]

[2] Nicotinamide + MNA + 2-Py + 4-Py.

[3] Kynurenic acid.

(HPLC) methods of Shibata[5,6] and those of nicotinamide, 2-Py, and 4-Py were simultaneously measured by the HPLC method of Shibata *et al.*[3]

Injection of a large amount of pyrazinamide did not affect the daily body weight gain or food intake, as shown in Table I.

Urinary excretion of nicotinamide, MNA, 2-Py, and 4-Py before and after injection of a large amount of pyrazinamide is also given in Table I. On day 1, the excretion of nicotinamide, MNA and 4-Py each increased by around 10-fold, and the urinary excretion of 2-Py by around 36-fold compared with the day before injection. This contrasts with the finding by Nasu *et al.*[1] that quinolinic acid excretion increased greatly, but the excretion of nicotinamide and MNA was not significantly increased. This might be mainly attributable to the difference in the period of urine collection between Nasu's experiment[1] (urine was collected for 6 hr after injection of tryptophan followed by intraperitoneal administration of pyrazinamide) and upon experiment (urine was collected for 24 hr after injection of pyrazinamide), because pyrazinamide itself inhibits poly(ADP-ribose) synthetase *in vitro*, which catalyzes the reaction $n\cdot(\text{NAD})\rightarrow n$ nicotinamide + $(\text{ADP-ribose})_n$ at higher concentrations.[1] If this is the case *in vivo*, the reaction NAD→nicotinamide might be inhibited only during the initial period after pyrazinamide loading, because the administered pyrazinamide could be quickly metabolized and/or excreted into urine. On the other hand, this inhibition could be taken away in our experiment because the period of urine collection was much longer than in Nasu's experiment.[1] Another possible explanation is that the quinolinic acid–nicotinamide conversion needs a time much longer than the tryptophan–quinolinic acid conversion.

The 2-Py excretion increased by 36-fold (Table I), but this might include pyrazinamide, which is co-eluted with 2-Py under the analytical conditions used. There is the possibility that the large increases in nicotinamide and its metabolites are due to coexisting pyrazinamide metabolites. However, this possibility would be low because the increased ratio of nicotinamide, MNA, and 4-Py each was almost the same. On days 2 and 3 after injection, urinary excretion of nicotinamide, MNA, 2-Py, and 4-Py were still over that in the normal state before injection. By days 4 and 5, these values had returned to normal. Therefore, it was found that the acceleration of tryptophan-niacin conversion by pyrazinamide administration would continue for 2–3 days.

It is reported that exogenous quinolinic acid administration contributes little to the increase in the urinary excretion of MNA.[7-9] All nicotinamide and its metabolites such as MNA, 2-Py and 4-Py are synthesized only from tryptophan in this experiment, so the increased excretion of these compounds means that endogenous quinolinic acid is efficiently metabolized into nicotinamide *via* NAD. This supports the hypothesis that the extremely low quinolinic acid-MNA conversion when quinolinic acid is administered exogenously to rats[7-9] is due to the poor penetration of quinolinic acid into cells.[10] Furthermore, this finding suggests that the limiting factor in the reaction quinolinic acid→nicotinic acid mononucleotide is quinolinic acid, not quinolinate phosphoribosyltransferase, 5-phosphoribosyl 1-pyrophosphate, or ATP.

The urinary excretion of kynurenic acid did not change when pyrazinamide was injected into rats as shown in Table I. This suggests that pyrazinamide did not affect the reactions of tryptophan→*N*-formylkynurenine→kynurenine→kynurenic acid.

In conclusion, it was found that pyrazinamide administration greatly increases the tryptophan–niacin conversion. Furthermore, our results support the claim that the tryptophan–niacin conversion is mainly due to the activity of ACMSDase.[11-13]

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