Effect of Prednisolone on the Urinary Excretion of Nicotinamide and Its Metabolites in Rats Fed with a Niacin-free Diet

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Adrenal cortical hormone seems to play an important role in *de novo* NAD biosynthesis from tryptophan. Therefore, the effects of prednisolone, a synthetic adrenal cortical hormone, on the urinary excretion of nicotinamide and such catabolic metabolites as *N*¹-methylnicotinamide (MNA), *N*¹-methyl-2-pyridone-5-carboxamide (2-Py) and *N*¹-methyl-4-pyridone-3-carboxamide (4-Py) were investigated. Rats fed with a niacin-free diet were intraperitoneally injected with prednisolone (3 mg/rat) three times at 09:00, 13:00 and 17:00 hr on day 0. As a result, the urinary excretion of nicotinamide, MNA, 2-Py and 4-Py was each higher in the prednisolone group than in the physiological saline group (for control) after 1 day; however, each excretion was lower in the prednisolone group than in control group after 3, 4, 5 and 6 days. On around the 7th day, the urinary excretion in the prednisolone group was restored to the control values. The initial rapid increase and subsequent decrease in the urinary excretion of nicotinamide and its metabolites by an injection of prednisolone is considered to be attributable to a respective elevation of the liver tryptophan oxygenase level and the liver aminocarboxymuconate-semialdehyde decarboxylase level.

The pituitary-adrenal system seems to play an important role in *de novo* NAD biosynthesis from tryptophan (Trp): [1] a glucocorticoid hormone load caused a rapid elevation of Trp oxygenase level¹⁾; [2] only in hypophysectomized rats, aminocarboxymuconate-semialdehyde decarboxylase (ACMSDase) activity was elevated by an injection of adrenal cortical hormone²⁾; [3] ACMSDase activity decreased upon an adrenalectomy.³⁾

Sanada and Miyazaki⁴⁾ have reported that an injection of prednisolone, a synthetic adrenal cortical hormone which has potent glucocorticoid activity but no mineralocorticoid activity,⁵⁾ caused an increase in hypophysectomized rat liver ACMSDase activity, which plays an important role in *de novo* NAD biosynthesis from Trp. Higher ACMSDase activity generally means a lower formation of quinolinic acid and such metabolites as nicotinamide (Nam), *N*¹-methylnicotinamide (MNA), *N*¹-methyl-2-pyridone-5-carboxamide (2-Py) and *N*¹-methyl-4-pyridone-3-carboxamide (4-Py). On the other

hand, Blake and Kun⁶⁾ have reported that an injection of hydrocortisone, an adrenal cortical hormone, caused an increase in liver Trp oxygenase activity, which is the initial enzyme in *de novo* NAD biosynthesis. Higher Trp oxygenase activity generally means a higher formation of quinolinic acid and its metabolites. This paper reports the effects of an injection of prednisolone on the urinary excretion of Nam, MNA, 2-Py and 4-Py from rats fed with a niacin-free diet.

Materials and Methods

Chemicals. MNA chloride was purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). 2-Py and 4-Py were synthesized by the methods of Pullman and Colowick⁷⁾ and Shibata *et al.*,⁸⁾ respectively. Prednisolone was purchased from Wako Pure Chemical Industries (Osaka, Japan). All other chemicals used were of the highest purity available from commercial sources.

Animals and diet. Male rats of the Wistar strain (experiment I, 3-weeks old; experiment II, 8-weeks old) were purchased from Clea Japan Inc. (Tokyo, Japan). The rats were immediately placed in individual metabolic cages

Table I. COMPOSITION OF THE DIET

	g/kg of diet
Vitamin-free casein ^a	200
L-Methionine ^a	2
α -Cornstarch ^b	458
Sucrose ^a	230
Corn oil ^c	50
Mineral mixture ^d (Oriental's ratio)	50
Vitamin mixture ^e (Oriental's ratio, nicotinic acid-free)	10

^a Obtained from Wako Pure Chemical Industries (Osaka, Japan).

^b Obtained from Nichiden Kagaku Co. (Osaka, Japan).

^c Obtained from Ajinomoto Co. (Tokyo, Japan).

^d Obtained from Oriental Yeast Kogyo Co. (Tokyo, Japan). The composition is described in ref. 11.

^e Obtained from Oriental Yeast Kogyo Co. (Tokyo, Japan). The composition is described in ref. 11.

(CT-10, obtained from Clea Japan) and fed with a nicotinic acid-free and 20% casein diet (Table I) *ad libitum* for 64 days (experiment I) and for 38 days (experiment II). This diet did not contain niacin; however, sufficient niacin can be synthesized from Trp in casein. In experiment I, rats ($n=5$) were intraperitoneally injected with 3 mg of prednisolone suspended in 1 ml of sterilized saline/rat three times at 09:00, 13:00 and 17:00 hr on the 53rd day. In experiment II, rats ($n=5$) were intraperitoneally injected with 3 mg of prednisolone suspended in 1 ml of sterilized saline/rat three times at 09:00, 13:00 and 17:00 hr on the 32nd day. As a control, rats ($n=5$) were intraperitoneally injected with 1 ml of sterilized saline/rat three times at 09:00, 13:00 and 17:00 hr on the 32nd day. Urine samples were collected as indicated in Table II (experiment I) and Table III (experiment II).

The room temperature was kept at $22 \pm 2^\circ\text{C}$ with a humidity of about 60%, and a 12-hr light/dark cycle was maintained. Body weight and food intake were measured daily at around 09:00 after the injection of prednisolone.

Analyses. Urine values for Nam, 2-Py and 4-Py were simultaneously measured by the high-performance liquid chromatographic (HPLC) method of Shibata *et al.*⁸⁾ Urine values for MNA were measured by the HPLC method of Shibata.⁸⁾

Results

Experiment I

Figure 1 shows the effect of prednisolone on the urinary excretion of Nam, MNA, 2-Py and 4-Py in rats fed with a niacin-free diet.

Fig. 1. Effect of Prednisolone on the Urinary Excretion of Nam (A), MNA (B), 2-Py (C) and 4-Py (D) (Experiment I).

For the experimental conditions, see Table II. Values are means $\pm$ SEM for five rats.

Prednisolone (3 mg/rat) was injected into the rats three times at 09:00, 13:00 and 17:00 hr. The day of injection was designated day 0. Under these conditions, Nam, MNA, 2-Py and 4-Py were synthesized from only Trp. The urinary excretion of Nam, MNA, 2-Py and 4-Py each tended to increase during the 1st day; however, each excretion decreased during the 3rd and 4th days as compared with the pre-injection day (control values for day -1). These minimum values were observed on the 3rd day and 4th day, and each excretion had tended to revert to the control values thereafter. Each excretion had recovered to the pre-injection value by the 9th day.

Table II. EFFECT OF PREDNISOLONE ON THE TRYPTOPHAN-NIACIN CONVERSION FACTOR (EXPERIMENT I)

Male rats of the Wistar strain (3 weeks old) were fed with a nicotinic acid-free and 20% casein diet (see Table I) *ad libitum* for 64 days. The rats were then intraperitoneally injected with 3 mg of prednisolone suspended in 1 ml of sterilized saline/rat at 09:00, 13:00 and 17:00 hr on the 53rd day of the experiment. Urine samples were collected from 09:00 hr on the 52nd day to 09:00 hr on the 53rd day (day -1), from 09:00 hr on the 53rd day to 09:00 hr on the 54th day (day 1), from 09:00 hr on the 54th day to 09:00 hr on the 55th day (day 2), from 09:00 hr on the 55th day to 09:00 hr on the 56th day (day 3), from 09:00 hr on the 56th day to 09:00 hr on the 57th day (day 4), from 09:00 hr on the 61st day to 09:00 hr on the 62nd day (day 9) and from 09:00 hr on the 64th day to 09:00 hr on the 65th day (day 12).

	Prednisolone
Day -1	
Body weight gain (g/day)	2.5 ± 0.3
Food intake (g/day)	17.0 ± 0.9
Trp intake (μmol/day) ^a	196 ± 10
(Nam + MNA + 2-Py + 4-Py) excretion (μmol/daily urine)	4.56 ± 0.96
Trp conversion factor ^b	51.8 ± 11.1
Day 1	
Body weight gain (g/day)	-11.6 ± 0.5
Food intake (g/day)	13.6 ± 0.7
Trp intake (μmol/day)	157 ± 8
(Nam + MNA + 2-Py + 4-Py) excretion (μmol/daily urine)	5.73 ± 1.10
Trp conversion factor	32.0 ± 6.2
Day 2	
Body weight gain (g/day)	-9.5 ± 0.6
Food intake (g/day)	9.4 ± 0.7
Trp intake (μmol/day)	108 ± 8
(Nam + MNA + 2-Py + 4-Py) excretion (μmol/daily urine)	3.68 ± 0.56
Trp conversion factor	31.5 ± 4.1
Day 3	
Body weight gain (g/day)	5.1 ± 1.6
Food intake (g/day)	12.5 ± 0.9
Trp intake (μmol/day)	144 ± 10
(Nam + MNA + 2-Py + 4-Py) excretion (μmol/daily urine)	1.35 ± 0.17
Trp conversion factor	110.3 ± 11.1
Day 4	
Body weight gain (g/day)	6.5 ± 1.6
Food intake (g/day)	14.9 ± 0.8
Trp intake (μmol/day)	171 ± 9
(Nam + MNA + 2-Py + 4-Py) excretion (μmol/daily urine)	2.03 ± 0.3

Table II. (continued).

	Prednisolone
Trp conversion factor	91.4 ± 10.8
Day 9	
Body weight gain (g/day)	2.2 ± 0.4
Food intake (g/day)	17.8 ± 1.3
Trp intake (μmol/day)	205 ± 15
(Nam + MNA + 2-Py + 4-Py) excretion (μmol/daily urine)	4.99 ± 0.94
Trp conversion factor	46.4 ± 7.5
Day 12	
Body weight gain (g/day)	1.9 ± 0.4
Food intake (g/day)	17.1 ± 1.1
Trp intake (μmol/day)	197 ± 12.7
(Nam + MNA + 2-Py + 4-Py) excretion (μmol/daily urine)	4.29 ± 0.85
Trp conversion factor	51.9 ± 8.2

^a Trp intake (μmol/day) = [food intake (g/day) × 0.903 × 0.013 × 0.2 × 10⁶] / 204. 0.903, protein content in casein; 0.013, Trp content in casein; 0.2, casein content in the diet.

^b Trp conversion factor = [Trp intake (μmol/day)] / [(Nam + MNA + 2-Py + 4-Py) excretion (μmol/day)]. Values are means ± SEM for five rats.

Table II shows the effects of prednisolone on the body weight gain, food intake, Trp intake, sum of Nam, MNA, 2-Py and 4-Py excretion, and Trp conversion factor [Trp intake (μmol/day) / (Nam + MNA + 2-Py + 4-Py excretion) in μmol/daily urine]. Body weight decreased during the 1st and 2nd days after the prednisolone injection, and increased again thereafter. Food intake also decreased during the 1st, 2nd and 3rd days after the injection as compared with the pre-injection day. The urinary excretion of the sum of Nam, MNA, 2-Py and 4-Py tended to be higher during the 1st day than in day -1 (control value). However, this value decreased after 2, 3 and 4 days as compared with the control. The minimum value of the sum of Nam, MNA, 2-Py and 4-Py excretion was observed on the 3rd day. By the 9th day, this value had recovered to the control level. The Trp conversion factor under normal conditions is around 50 (day -1, and days 9 and 12). The lowest Trp conversion factor, namely the highest Nam and its me-

tabolite formation from Trp, was observed at days 1 and 2 (around 30), and the highest Trp conversion factor, namely the lowest Nam and its metabolite formation from Trp, was observed at day 3 (around 110).

Experiment II

The experiment was repeated to determine the initial increase and subsequent decrease in the urinary excretion of Nam and its metabolites by an injection of prednisolone. This time, the age of the rats and the feeding period of the niacin-free diet were selected differently to check that the prednisolone effects were not

Fig. 2. Effect of Prednisolone on the Urinary Excretion of Nam (A), MNA (B), 2-Py (C) and 4-Py (D) (Experiment II).

For the experimental conditions, see Table III. $\circ$, control group; $\bullet$, prednisolone group. Values are means $\pm$ SEM for five rats.

Table III. EFFECT OF PREDNISOLONE ON THE TRYPTOPHAN-NIACIN CONVERSION FACTOR (EXPERIMENT II)

Male rats of the Wistar strain (8 weeks old) were fed with a nicotinic acid-free and 20% casein diet (see Table I) *ad libitum* for 38 days. The rats were intraperitoneally injected with 3 mg of prednisolone suspended in 1 ml of sterilized saline/rat at 09:00, 13:00 and 17:00 hr on the 32nd day of the experiment. As a control, the rats were injected with 1 ml of sterilized saline at 09:00, 13:00 and 17:00 hr on the 32nd day. Urine samples were collected from 09:00 hr on the 31st day to 09:00 hr on the 32nd day (day -1), from 09:00 hr on the 32nd day to 09:00 hr on the 33rd day (day 1), from 09:00 hr on the 33rd day to 09:00 hr on the 34th day (day 2), from 09:00 hr on the 34th day to 09:00 hr on the 35th day (day 3), from 09:00 hr on the 35th day to 09:00 hr on the 36th day (day 4), from 09:00 hr on the 36th day to 09:00 hr on the 37th day (day 5), from 09:00 hr on the 37th day to 09:00 hr on the 38th day (day 6), and from 09:00 hr on the 38th day to 09:00 hr on the 39th day (day 7).

	Control	Prednisolone
Day -1		
Body weight gain (g/day)	1.5 $\pm$ 0.2	1.3 $\pm$ 0.2
Food intake (g/day)	14.3 $\pm$ 0.8	15.4 $\pm$ 0.3
Trp intake ($\mu\text{mol/day}$) ^a	165 $\pm$ 9	177 $\pm$ 3
(Nam + MNA + 2-Py + 4-Py) excretion ($\mu\text{mol/daily urine}$)	3.86 $\pm$ 0.62	3.68 $\pm$ 0.42
Trp conversion factor ^b	50.0 $\pm$ 9.9	50.5 $\pm$ 5.9
Day 1		
Body weight gain (g/day)	1.3 $\pm$ 0.2	-8.3 $\pm$ 0.9
Food intake (g/day)	15.4 $\pm$ 0.7	16.8 $\pm$ 0.8
Trp intake ($\mu\text{mol/day}$)	177 $\pm$ 8	193 $\pm$ 9
(Nam + MNA + 2-Py + 4-Py) excretion ($\mu\text{mol/daily urine}$)	3.41 $\pm$ 0.36	4.63 $\pm$ 0.35
Trp conversion factor	55.7 $\pm$ 5.7	42.2 $\pm$ 1.7
Day 2		
Body weight gain (g/day)	1.5 $\pm$ 0.3	-10.6 $\pm$ 0.9
Food intake (g/day)	16.2 $\pm$ 1.1	9.1 $\pm$ 1.1
Trp intake ($\mu\text{mol/day}$)	186 $\pm$ 12	105 $\pm$ 13
(Nam + MNA + 2-Py + 4-Py) excretion ($\mu\text{mol/daily urine}$)	3.95 $\pm$ 0.32	4.48 $\pm$ 0.28
Trp conversion factor	50.0 $\pm$ 2.7	23.7 $\pm$ 3.0
Day 3		
Body weight gain (g/day)	2.1 $\pm$ 0.3	1.6 $\pm$ 1.6
Food intake (g/day)	17.2 $\pm$ 0.7	11.3 $\pm$ 1.3
Trp intake ($\mu\text{mol/day}$)	198 $\pm$ 8	130 $\pm$ 15
(Nam + MNA + 2-Py + 4-Py) excretion ($\mu\text{mol/daily urine}$)	3.70 $\pm$ 0.32	2.33 $\pm$ 0.12
Trp conversion factor	55.6 $\pm$ 3.3	55.4 $\pm$ 5.1
Day 4		
Body weight gain (g/day)	1.2 $\pm$ 0.3	4.4 $\pm$ 1.0
Food intake (g/day)	14.7 $\pm$ 1.4	14.8 $\pm$ 1.1

Table III. (continued).

	Control	Prednisolone
Trp intake ($\mu\text{mol/day}$)	169 $\pm$ 16	170 $\pm$ 13
(Nam + MNA + 2-Py + 4-Py) excretion ($\mu\text{mol/daily urine}$)	3.45 $\pm$ 0.38	1.58 $\pm$ 0.10
Trp conversion factor	54.7 $\pm$ 5.1	108.6 $\pm$ 10.4
Day 5		
Body weight gain (g/day)	1.5 $\pm$ 0.3	2.3 $\pm$ 0.8
Food intake (g/day)	14.0 $\pm$ 2.1	15.6 $\pm$ 0.5
Trp intake ($\mu\text{mol/day}$)	161 $\pm$ 24	179 $\pm$ 6
(Nam + MNA + 2-Py + 4-Py) excretion ($\mu\text{mol/daily urine}$)	3.66 $\pm$ 0.21	1.57 $\pm$ 0.09
Trp conversion factor	47.4 $\pm$ 4.2	116.0 $\pm$ 9.6
Day 6		
Body weight gain (g/day)	1.3 $\pm$ 0.2	1.9 $\pm$ 0.3
Food intake (g/day)	15.5 $\pm$ 1.9	18.0 $\pm$ 0.6
Trp intake ($\mu\text{mol/day}$)	178 $\pm$ 22	207 $\pm$ 7
(Nam + MNA + 2-Py + 4-Py) excretion ($\mu\text{mol/daily urine}$)	3.94 $\pm$ 0.45	2.26 $\pm$ 0.08
Trp conversion factor	43.5 $\pm$ 5.1	92.4 $\pm$ 5.9
Day 7		
Body weight gain (g/day)	1.5 $\pm$ 0.2	1.3 $\pm$ 0.2
Food intake (g/day)	14.6 $\pm$ 1.7	15.3 $\pm$ 1.6
Trp intake ($\mu\text{mol/day}$)	168 $\pm$ 19	176 $\pm$ 18
(Nam + MNA + 2-Py + 4-Py) excretion ($\mu\text{mol/daily urine}$)	3.89 $\pm$ 0.55	2.86 $\pm$ 0.23
Trp conversion factor	44.5 $\pm$ 4.5	63.7 $\pm$ 8.3

^a Trp intake ($\mu\text{mol/day}$) = [Food intake (g/day) $\times$ 0.903 $\times$ 0.013 $\times$ 0.2 $\times$ 10⁶] / 204. 0.903, protein content in casein; 0.013, Trp content in casein; 0.2, casein content in the diet.

^b Trp conversion factor = [Trp intake ($\mu\text{mol/day}$) / [(Nam + MNA + 2-Py + 4-Py) excretion ($\mu\text{mol/day}$)]. Values are means $\pm$ SEM for five rats.

related to these parameters. Figure 2 shows the effects of prednisolone on the urinary excretion of Nam, MNA, 2-Py and 4-Py. The rats were injected with prednisolone (3 mg/rat) or physiological saline three times at 09:00, 13:00 and 17:00 hr on day 0. Each excretion was higher in the prednisolone group than in the physiological saline group (control group) on the 1st and 2nd days after the injection. However, each excretion was lower in the prednisolone group than in the control group on the 3rd, 4th and 5th days. On the 6th and 7th days, each excretion tended to revert to the pre-injection values (day -1). Each urinary excretion in the control group was almost

constant during the experiment.

Table III shows the effects of prednisolone on the body weight gain, food intake, Trp intake and Trp conversion factor. Body weight was decreased on the 1st and 2nd days after the injection, and increased again on the 3rd day and thereafter. Food intake in the prednisolone group was also decreased on the 1st and 2nd days as compared with the control group. The urinary excretion of the sum of Nam, MNA, 2-Py and 4-Py was higher in the prednisolone group than in the control group on the 1st and 2nd days; however, it was lower in the prednisolone group than in the control group on the 3rd, 4th and 5th days. From the 6th day, it tended to revert to the pre-injection level (day -1). The Trp conversion factor under normal conditions is around 50. This conversion factor was lower in the prednisolone group than in the control group on the 2nd day after the injection; namely, the conversion of Nam from Trp was increased by the injection. The highest Trp conversion factor in the prednisolone group, namely the lowest niacin formation from Trp, was observed on the 4th and 5th days. On the 7th day, these values had almost recovered to the pre-injection value.

Discussion

Prednisolone was injected into normal rats three times at 09:00, 13:00 and 17:00 hr on day 0 of the experiment. An injection of prednisolone caused an increase in niacin formation from Trp on the 1st day after the injection. However, its formation decreased from the 3rd to 6th days after the injection. The effects of prednisolone had disappeared by around the 7th day after the injection. Blake and Kun⁶⁾ have reported that after rats had been injected with hydrocortisone (5.2×10^{-2} nmol/kg of body weight) and then sacrificed 6 hr subsequently, the liver Trp oxygenase activity increased about 9-fold. Sanada and Miyazaki⁴⁾ reported that after hypophysectomized rats had been injected with prednisolone (2.5 mg/rat) four times at 12-hr intervals and

sacrificed 12-hr after the last injection, the liver Trp oxygenase activity did not change as compared with the control up to 84 hr after the last injection. Furthermore, Sanada and Miyazaki⁴) reported that after hypophysectomized rats had been injected with both prednisolone (2.5 mg/rat) and somatotropin (a pituitary hormone, 1 mg/rat) four times at 12-hr intervals and sacrificed 12-hr, 36-hr and 84-hr after the last injection, the liver ACMSDase activity increased with time to a maximum value after 84 hr (about 7-fold higher than in the control). Hayakawa and Iwai¹⁰) reported that after adrenalectomized rats had been injected with prednisolone (3 mg/rat) twice at 16:00 and 01:00 hr and sacrificed at 09:30 hr, the liver ACMSDase activity increased, although its increase was slight (about 2-fold) as compared with the control. These findings,^{4,6,10}) namely, a rapid elevation of Trp oxygenase level and slow elevation of ACMSDase level by adrenal cortical hormones, are consistent with the present results; rapid increases in the Nam, MNA, 2-Py and 4-Py excretion and subsequent decreases in each excretion thereafter were observed when prednisolone was injected into the rats. In other words, the initial increase and subsequent decrease in the urinary excretion of Nam and its metabolites is considered to be attributable to an elevation of Trp oxygenase level and an elevation of ACMSDase level, respectively. Accordingly, it was found that niacin formation from Trp increased initially, decreased

subsequently, and reverted to its original level when prednisolone was injected into the rats.

The Trp conversion factor is considered to have affected the body weight change of the rats: when rats gain body weight, Trp and Nam are retained in the body; on the other hand, when rats lose body weight, Trp and Nam are released from the body. However, it is not clear how much Trp and Nam are retained in body when rats gain 1 g of body weight, and how much Trp and Nam are released from the body when rats lose 1 g of body weight. Therefore, this must be elucidated to calculate the real Trp conversion factor.

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