METABOLISM OF PANTETHINE IN THE RAT

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The consecutive changes in blood concentrations and the urinary excretion of free and bound pantothenic acid were studied after oral and intravenous administration of pantethine (21.6 μmoles/kg) to rats. The results were compared with those obtained after administration of Ca-pantothenate (21.6 μmoles/kg).

The concentration of total pantothenic acid in blood was significantly higher in the pantethine group than in the Ca-pantothenate group after oral administration of vitamins. The maximal concentrations were observed at 2–4.5 hr after ingestion in both groups. The excretion rates of total pantothenic acid in 24-hr urines were 29±3% of the dose and 18±2% in the pantethine and the Ca-pantothenate groups, respectively. These findings indicate that pantethine was more absorbable through the gastrointestinal wall of the rat than Ca-pantothenate. No significant difference was, however, found in the amounts of bound pantothenic acid in blood between these two groups. Pantethine was found to be hydrolyzed, to some extent, to pantothenic acid by an enzyme during passing the intestinal mucosa.

After an intravenous injection of pantethine, about 50% of total pantothenic acid in blood was pantethine and the rest was free pantothenic acid, while little bound pantothenic acid was detected after injection of Ca-pantothenate. The decrease in total pantothenic acid in blood was significantly delayed in the early period after injection in the pantethine group when compared with that of the Ca-pantothenate group, although both the vitamins were almost completely eliminated in 24-hr urines after injection.

The metabolic fate of panteth(e)ine from exogeneous sources is directed toward either biosynthesis to CoA or degradation to pantothenic acid in animal cells. The biosynthesis of CoA from panteth(e)ine has been evidenced in vitro by the fact that panteth(e)ine is easily phosphorylated by pantothenate kinase to join the main pathway to CoA (1-4). When administered parenterally to rats having a reduced CoA level, panteth(e)ine can more efficiently restore the hepatic

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CoA level than pantothenate (5, 6). On the other hand, it has been reported that panteth(e)ine undergoes in vitro hydrolysis to pantothenic acid with \( \beta \)-mercaptoethylamine as another product by the action of a specific amidohydrolase, one of the member enzymes involved in the metabolic dissimilation of CoA (4, 7-9).

However, in spite of the extensive studies in vitro, little is known about the gastrointestinal absorption and the urinary excretion of pantethine or its metabolites after administration to animals including human subjects. A few studies have been reported among them SHIGETA and SHICHIRI (10) and YOSHIMURA et al. (11) who studied the urinary excretion of pantethine and its metabolites in human subjects and rabbits, respectively, after parenteral administration of the vitamin.

The present report deals with the consecutive changes in the blood concentration and the urinary excretion of pantothenic acid of free and bound forms in rats after oral and intravenous administration of pantethine.

In order to obtain a more detailed insight into the intestinal absorption of pantethine, some additional experiments have also been performed.

MATERIALS AND METHODS

1. Administration of drugs. In the experiment for oral administration, 15 male rats of Wistar strain weighing 140 to 160 g were used. The rats were divided into 3 groups of 5 rats each. The rats of the first group were administered 12 mg (21.6 \( \mu \)moles) of pantethine (PTSS) in 2 ml of water per kg body weight with a stomach tube, those of the second group with 10.28 mg (21.6 \( \mu \)moles) of Ca-pantothenate (Ca-PaA) per kg, and those of the last group with 2 ml of water to serve as the control.

The experiment for intravenous administration was carried out with 9 male Wistar rats weighing 200 to 250 g. These rats were divided into 3 groups consisting of 3 rats each. The rats of the first, the second and the third groups were injected via the femoral vein with PTSS (12 mg/ml saline/kg), Ca-PaA (10.28 mg/ml saline/kg) and saline (1 ml/kg), respectively.

2. Collection of blood, urine and liver samples. Two blood samples of 100\( \mu \)l each were taken from the tail vain at the time indicated in the text. The samples were put into small test tubes containing 50 \( \mu \)l of 0.05 M Tris-HCl buffer (pH 8.3). The one sample was used for the determination of free PaA, and the other was used for the assay of total PaA.

Urine samples were collected before and at 24-hr intervals after administration of the drugs, filtered through glass filter paper (Toyo Roshi Co., Tokyo, Type DP-25) and assayed for free and total PaA.

A piece of liver, weighing one gram, was excised from the rat 24 hr after intravenous administration of the drugs and was homogenized with 4 ml of cold
0.1 M acetate-HCl buffer (pH 5.3). The homogenate was deprotenized by the method described by Soyama (12). The supernatant was used for the assay of free and total PaA.

3. Measurement of the rate of intestinal absorption in situ. This was carried out according to Aso et al. (13). Male rats of Wistar strain weighing 180 to 190 g were made to fast overnight and were anesthetized by an intraperitoneal injection of pentobarbital (40 mg per kg). A gauze pad moistened with a warm (37°C) saline was placed on the lower part of the ileum exposed by midline incision.

The mesenteric arteries and veins were ligated at the sites indicated in Fig. 1, a and b. The rat was then heparinized with an injection of Na-heparin (Daiichi Pure Chemicals Co., Tokyo, 1,000 U/ml saline/kg) via the femoral vein. At about 3 min after heparinization, the vena mesenterica cranialis (Fig. 1, c) was ligated and cannulated with a polyethylene tubing (1 mm the inside diameter) at the distal side of the ligature for collection of blood. The ileum was ligated at 2 sites: the distal end and a proximal site about 5 cm apart from it (Fig. 1, d and e).

![Fig. 1. Diagram showing the procedure of canulation of the rat mesenteric vein for the study on the intestinal absorption of pantethine and Na-pantothenate in situ. X, Site of ligation.](image)

PTSS (21.6 μmoles/2 ml saline/kg) or Na-pantothenate (43.2 μmoles/2 ml saline/kg) was infused into the ligated loop of the ileum. pH of the drug solutions was 6.2. Blood was successively collected in test tubes at 5 minute-intervals after intraileum infusion of the drug solution. The flow rate of blood was approximately 2 ml/5 min. During the experiment the rat was transfused via the femoral vein with the heparinized whole blood which had been previously collected from other rats.

4. Assay of free and total PaA. PaA was determined microbiologically with Lactobacillus arabinosus 17–5 as described previously (3). The assay of
PaA with this strain was not affected by PTSS when the molar ratio of PTSS to PaA in assay samples was less than 0.7.

Total PaA was determined microbiologically after treatment of samples with pigeon-liver enzyme (amidase) and calf intestinal alkaline phosphatase according to the method of NOVELLI (14). The pigeon-liver enzyme was prepared by the KAPLAN-LIPMANN method (15) and was treated with Dowex-1 to remove endogenous CoA (14). The reaction mixture for the enzyme treatment contained 50 μl of the pigeon-liver enzyme, 50 μl of calf intestinal alkaline phosphatase (Boehringer Mannheim Co., Ltd. 0.5 units), 0.75 μg reduced glutathione, 50 μl of 0.5 M Tris-HCl, pH 8.3, and 100 μl of a test sample (whole blood, appropriately diluted urine and liver extract). After incubation for 2 hr at 37°C, the mixture was deprotenized according to the method described by MASUGI (16). The precipitate was washed with 2.5 ml of water and then the combined supernatant was assayed for PaA.

For determination of free PaA, 100 μl of sample was added to 50 μl of Tris-HCl buffer and 100 μl of water and then the supernatant for PaA assay was immediately prepared as described above without the incubation.

The recovery experiment in this assay system showed 95 to 105% yield of PaA, PTSS, phosphopantetheine and CoA, separately or in combination, added to blood or urine. The recovery of the last two substances was extremely low (14 to 20%) when alkaline phosphatase was omitted from the assay system.

5. Assay of the pantethine splitting-activity of the intestinal mucosa. Fresh intestinal mucosal tissue of rats was obtained by squeezing the intestinal mucosal surface with a glass rod and 2.7 g of the tissue was homogenized with 2 ml of 0.25 M sucrose. The activity of a PTSS-splitting enzyme in the homogenate was assayed by the following system. 0.1 ml of PTSS (50 μmoles) or H2O, 0.1 ml of cysteine (20 μmoles), 0.3 ml of 0.2 M Tris-maleate buffer (pH 7.0) and 0.5 ml of the homogenate (26 mg protein per ml) were mixed. After incubation for 30 min at 37°C, this reaction mixture was boiled for 30 sec and then centrifuged. An aliquot of the supernatant was loaded on a Dowex-1 column (0.7 cm × 4 cm) and the column was washed with water. PaA, if present, was adsorbed on the resin was eluted with 0.1 M NaCl. The eluate was assayed for PaA.

RESULTS

1. Blood level and urinary excretion of PaA after oral administration of PaA or PTSS.

Table 1 shows the blood concentration of free and total PaA after oral administration of water, Ca-PaA or PTSS (PaA equivalent 43.2 μmoles per kg).

The increase in the free and total PaA concentrations was significantly more pronounced in the PTSS group than in the PaA group, and it reached maximum levels 2 hr after administration. However, no difference was observed between
these groups with respect to the amount of bound PaA which was calculated as a difference of the amounts of total and free PaA.

| No. of rats | 5 | 5 | 5 |
| Body weight (g) | 148±4 | 154±2 | 151±3 |

Table 1. Free and total pantothenic acid in blood before and after oral administration of pantethine and Ca-pantothenate to rats.

| Time after administration (hr) | Free PaA | Total PaA | Free PaA | Total PaA |
|-------------------------------|----------|-----------|----------|-----------|
| 0                             | 2.58±0.1 | 2.87±0.1  | 2.58±0.1 | 2.87±0.1  |
| 1                             | 2.43±0.06| 3.23±0.11| 3.07±0.11| 3.79±0.25a|
| 2                             | 2.34±0.07| 3.30±0.05| 4.06±0.16b| 4.82±0.09|
| 3                             | 2.09±0.04| 3.15±0.05| 3.95±0.16a| 4.74±0.07|
| 4.5                           | 2.15±0.13| 3.33±0.21| 4.13±0.10c| 4.77±0.16|
| 7.5                           | 2.35±0.12| 2.70±0.11| 2.97±0.15| 3.87±0.26|
| 24                            | 2.61±0.24| 2.65±0.09| 2.40±0.06| 3.20±0.09|

All figures for total PaA contents of PTSS and Ca-PaA groups except those obtained at 24 hr were significantly different (p<0.01) from those of the control group.

As shown in Fig. 2, the rate of urinary excretion of total PaA was higher in the PTSS group than in the other, and the urinary excretion of PaA lasted in 2 to 3 days in the former group. The amount of urinary PaA (24 hr) amounted to 29±3% and 18±2% of the dose in the PTSS and the PaA groups, respectively, suggesting the higher availability of PTSS. No bound PaA could be detected in the urine of both the groups.

2. Blood and liver levels and urinary excretion of PaA after intravenous administration

When PTSS was administered intravenously, the profile of blood concentration of free and total PaA was markedly different from that obtained with oral administration of the vitamin. In the rats given PTSS, about 50% of the total PaA present in blood was found to be in the bound form, as shown in Fig. 3. Bound PaA seemed to consist mainly of PTSS because the value was not affected by omitting alkaline phosphatase from the assay mixture (see MATERIALS AND METHODS). Figure 3 also shows a rapid disappearance of total and free PaA from blood; this was reflected by the high rate of urinary excretion of PaA which amounted to around 100% of the dose in 24 hr in both of the PTSS and PaA.
Fig. 2. Urinary excretion of pantothenic acid before and after an oral administration of pantethine and Ca-pantothenate. Pantethine or Ca-pantothenate was given orally to rats at a dose of 21.6 \( \mu \text{moles/kg} \). Control rats were given water, 2 ml/kg. At 24 hr intervals before and after administration, urine was collected for assay of pantothenic acid. Histogram shows means \( \pm \) S.E. from 5 rats. A, Control; B, the Ca-PaA group and C, the PTSS group.

Table 2. Excretion of pantothenic acid in 24-hr urine after intravenous injection of pantethine and Ca-pantothenate.

| Group  | No. of rats | Dose \( \mu \text{moles/kg} \) | PaA excreted in 24-hr urine (\( \mu \text{moles} \)) | \% of dose |
|--------|-------------|-------------------------------|---------------------------------|------------|
|        |             |                               | Free PaA | Total PaA | Free PaA | Total PaA |
| PTSS   | 3           | 21.6                          | 10.4±0.1 | 13.6±0.5 | 79       | 103       |
| Ca-PaA | 3           | 21.6                          | 11.2±0.6 | 13.1±1.0 | 87       | 99        |
| Control| 3           | 0                             | 2.2±0.3  | 2.9±0.4  | —        | —         |

groups (Table 2). A significant amount (about 20\% of total) of bound PaA was excreted in the urine from the rats given PTSS, while the value was about 10\% in the PaA group.

More detailed analysis of the data indicated that the rate of decrease in the total PaA concentration in blood was slightly but significantly delayed in the rats given PTSS as compared with those given Ca-PaA.

The hepatic PaA level was slightly elevated in both the groups at 24 hr after administration; the most part of the increment was contributed by bound PaA.
Fig. 3. Concentrations of free and total pantothenic acid in blood after an intravenous injection of pantethine and Ca-pantothenate to rats. PTSS or Ca-PaA was intravenously injected to rats a dose of 21.6 μmoles/kg. Blood samples were consecutively taken from the tail vein for assay of pantothenic acid. The results were subtracted with the control basal level. Each point represents means of 5 rats. Closed symbols are for the total PaA concentrations of the PTSS group (▲) and the Ca-PaA group (●), and open symbols are for the free PaA concentrations of the PTSS group (△) and the Ca-PaA group (○).

because the free PaA level in the liver of these groups was close to the control level (Table 3).

Table 3. Pantothenic acid contents of the rat liver at 24 hr after intravenous injection of pantethine and Ca-pantothenate.

| Group   | No. of rat | Dose μmoles/kg | PaA eq. nmoles/g. wet liver |
|---------|------------|----------------|-----------------------------|
|         |            |                | Free PaA                    | Total PaA | Bound PaA |
| PTSS    | 3          | 21.6           | 19.3 ± 0.7                  | 380 ± 4.0 | 361 ± 3.4^b |
| Ca-PaA  | 3          | 21.6           | 16.5 ± 0.4                  | 371 ± 8.1 | 354 ± 7.9^c |
| Control | 3          | 0              | 15.6 ± 0.6                  | 316 ± 8.9 | 301 ± 8.8 |

^a Calculated from the difference between free and total PaA contents.
^b p < 0.01, ^c p < 0.02.

3. Intestinal absorption of PaA and PTSS in situ
Further studies were undertaken to obtain more detailed insight in the
intestinal absorption of PTSS. PTSS (21.6 μmoles/kg) or Na-PaA (43.2 μmoles/kg) was injected into the ligated part of the ileum and blood samples were taken consecutively through the cannula inserted in the mesenteric vein distributed in the ligated part of the ileum (see MATERIALS AND METHODS). The blood samples were assayed for total and free PaA. The results are illustrated in Fig. 4.

![Fig. 4. Intestinal absorption of pantethine and Na-pantothenate in situ. PTSS or Na-PaA was infused into the ligated loop of the lower part of the ileum of the anesthesized rats. Blood samples were consecutively taken for assay of PaA through a cannula inserted into the mesenteric vein. Each point represents means (±S.E.) of 4 rats. ---: Total PaA and -----: free PaA. For the experimental conditions, see MATERIALS AND METHODS.](image)

No significant difference in the rate of absorption was found between PTSS and Na-PaA, although the former was slightly more absorbable. In the rats given PTSS, free PaA was found to amount as much as 80% of the total PaA content absorbed in blood. This result suggests that a considerable part of PTSS given was hydrolyzed to PaA during passing through the intestinal wall. This was supported by the findings that the potent pantetheine-splitting enzyme activity located in the intestinal mucosal cells (Table 4) and that no conversion of PTSS to PaA was detected in blood in vitro.

| Substrate       | PaA released (nmoles/ml, 30 min) |
|-----------------|----------------------------------|
| Control (H2O)   | 6.6                              |
| PTSS (50 nmoles/ml) | 44.8                           |
DISCUSSION

There have been several reports on the biosynthesis of CoA from pantethine in vitro (1, 3) and in vivo (5, 6, 17) and on the degradative metabolism of pantethine toward pantothenic acid in vitro (4, 7, 8). Pantethine was reported to be phosphorylated by pantothene kinase to pantetheine 4'-phosphate which joined the main pathway of CoA biosynthesis (3, 4). On the other hand, pantethine was found to be directly hydrolyzed to pantothenic acid and β-mercaptoethylamine in vitro by a specific amidohydrolase (4, 7, 8, 18), which is thought to be a member enzyme involved in the metabolic dissimilation of CoA in animal cells (4). The degradation of pantethine has been also indicated in vivo by some workers (10, 11). SHIGETA and SHICHIRI (10) compared urinary excretion after an intramuscular injection of pantethine with that after injection of Ca-pantothenate in healthy human subjects, and they reported that 1.51 mg (10.1% of the dose) of pantothenic acid and 0.36 mg (1.8%) of pantethine were eliminated in 6-hr urine after pantethine (20 mg) administration, while 3.63 mg (21.3%) of pantothenic acid without pantethine was excreted in 6-hr urine after Ca-pantothenate (20 mg) administration. From these results, they suggested higher reabsorbability in renal tubules and higher affinity to tissues of pantethine in comparison with pantothenic acid (10). YOSHIMURA et al. (11) studied urinary excretion and urinary metabolites after a subcutaneous injecting rabbits with 14C-pantethine (20 mg/kg, labelled at the β-alanine moiety), and indicated that 43–90% and 2–3% of the radioactivity given were eliminated in 24-hr and 24–48-hr urines, respectively. They (11) also demonstrated that the rabbits administered with a larger dose (500 mg/kg) of 14C-pantethine excreted over 80% of the radioactivity found in 24-hr urine, and that the radioactivity in the urine was composed of pantothenic acid (about 50%), unchanged pantethine (about 10%) and unidentified substances with a trace amount of β-alanine.

However, there has been no report, to our knowledge, on the intestinal absorption and the urinary excretion after oral administration of pantethine. The results presented here indicated that the urinary excretion rates of pantothenic acid after equimolar (21.6 μmoles/kg) oral administration of pantethine and Ca-pantothenate were 29±3% and 18±2% in 24-hr urines, respectively: a significantly 1.5 times higher rate was obtained with pantethine administration. This suggests a higher absorbability of pantethine through the gastrointestinal wall of the rat. No significant increase in pantothenic acid of bound form was found in the urine of both groups.

It may be questionable, however, to calculate the absorption rate of a natural substance such as vitamins from the rate of its urinary excretion in balance study. NAKAMURA (19) reported that when rats was intraperitoneally injected with a small amount of 14C-pantothenic acid (886 μg/kg) which was comparable to natural daily input from diet, 77% of the radioactivity injected was incorporated...
into the liver at 24 hr after injection, and that the clearance time of the radioactivity from the liver was about 96 hr. This suggests that most of the exogenous pantothenate enters into a pool or pools of CoA intermediates including pantothenate and CoA in the liver.

On the other hand, the present study indicated that intravenously injected Ca-pantothenate (10.28 mg/kg) as well as pantethine (12 mg/kg) was almost completely eliminated in the urine within 24 hr, although a small but significant increase (below 5% of the dose) in bound pantothenate was observed in the liver 24 hr after injection. YOSHIMURA et al. (11) also showed a similar rapid and high rate of the urinary excretion after injecting 14C-pantethine (20 or 500 mg/kg) subcutaneously into rabbits, as cited above. The discrepancy between the results of these studies may be caused by the difference in the dose used and may also suggest an overflow phenomenon of excess dosing. Therefore, it seems reasonable in the present study to calculate the absorption rates of vitamins from the rates of urinary excretion of them and their metabolites.

In the oral administration study, the level of total PaA in blood was also significantly higher in the PTSS group than in the PaA group. No significant difference was, however, found in the amounts of bound PaA in blood between these two groups, although the bound PaA concentration was slightly higher in the PTSS group. From the study on the intestinal absorption in situ, PTSS was found to be metabolized, to a considerable extent, to PaA during passing the intestinal wall. The PTSS-splitting activity in the intestinal mucosa evidenced here seems to be involved in this dissimilative reaction. The PTSS-splitting activity was reported to be distributed also in the liver and the kidney of rats (4, 7, 8) and to be located in lysosomes and microsomes of these tissues and in the cytoplasmic fraction of the kidney (4). This enzyme, a specific amidohydrolase, was described as contributing to the dissimilation of CoA in the cells of these tissues (4). But DOMSCHKE et al. (20) demonstrated considerable stability of pantetheine or pantetheine-containing fragment of CoA in the liver perfusion system of rats, although CoA itself was promptly broken down to pantetheine-containing fragments on the erythrocyte and the liver cell membranes during perfusion. PTSS was stable in blood in vitro. Therefore, the degradation of PTSS to PaA in the body after being absorbed seems to occur mainly in the kidney cells.

After the intravenous injection of PTSS in rats, over 50% of total PaA in blood was found to be bound PaA most part of which was PTSS or pantetheine, while little bound PaA was detected in the blood of the rats injected with Ca-PaA. The clearance of total PaA from blood was slightly, but significantly faster, in the PaA group than in the PTSS group. This difference in the rate of decrease in total PaA in blood in the early period (0.5-4 hr after injection) between these two groups seems to be related to the presence of a considerable amount of bound PaA in the blood of the PTSS group. These findings may suggest a higher affinity of PTSS to tissues or, as postulated by SHIGETA and SHICHIRI (10), a
higher reabsorbability of PTSS in the renal tubular system in comparison with PaA.

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