EFFECTS OF PENICILLAMINE ON THE CONTENTS OF B₆ VITAMERS OF THE MOUSE BRAIN

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Summary The contents of B₆ vitamers were measured in the brains of mice treated with DL- and D-PeA. When a single convulsant dose of DL-PeA was injected, PLP content was decreased, being accompanied with production of PLP-thiazolidine. The effect of DL-PeA on PLP content was evident far before the occurrence of the convulsions. The administration of PN together with DL-PeA prevented the onset of seizures and lessened the effect of DL-PeA on PLP content. The same amount of D-PeA did not invoke seizures, but caused a small but significant decrease in PLP content.

PeA is a potent chelating agent that is used in the treatment of Wilson's disease and certain heavy metal intoxications (1, 2). It is currently under study in macroglobulinemia, the cold agglutinin syndrome, rheumatoid arthritis, and cystinuria (3–6).

It has been well established that L-PeA or DL-PeA exerts an antivitamin B₆ effect in the rat (7–9). This is manifest by growth depression, excessive urinary excretion of xanthurenic acid, and a reduced activity of PLP-dependent enzymes. All of these effects were reversed by the simultaneous administration of vitamin B₆. D-PeA was not growth inhibitory in the rat. Hence it has been believed to be devoid of antivitamin B₆ activity (9, 10). Moreover, an injection of large amounts of DL-PeA to mice was shown to cause attacks of convulsions which was not produced by D-PeA and was prevented by an injection of PN (11). It was also reported that the DL-PeA-treated animals showed a decreased activity of brain glutamic decarboxylase, and that this effect could also be reversed by the administration of PN (11).

Abbreviations used: PeA, penicillamine; PLP, pyridoxal phosphate; PN, pyridoxine; PMP, pyridoxamine phosphate; PL, pyridoxal; PM, pyridoxamine; PLP-thiaz, PLP-thiazolidine; PL-thiaz, PL-thiazolidine.
L-PeA was shown to form a thiazolidine compound with PLP (12, 13). Subsequent studies, however, indicated that D-PeA also formed a thiazolidine with PLP (14); hence it too might be expected to have antivitamin B$_6$ effect. In fact, an antagonism of D-PeA to vitamin B$_6$ has been demonstrated, using the urinary excretion of xanthurenic acid as a parameter of vitamin B$_6$ deficiency (15). The present studies were undertaken to test the effect of DL- and D-PeA on the distribution of B$_6$ vitamers in mouse brain which have previously been shown to reflect the deficiency of vitamin B$_6$ (16).

METHODS

Animals. DDY mice (weighing approx. 20 g) were used in this experiment.

Administration of drugs. Solutions of PeA and PN (Nakarai Chemicals) were prepared daily in 0.9% saline, the pH being adjusted to 7.0 immediately before use. The final concentration of the drugs was adjusted so that the required dosage was administered in a volume equivalent to 1% of the body weight of the animals. Injections of PeA and PN were intraperitoneal and intramuscular, respectively, and the injected animals were kept in a laboratory with minimal background noise.

Determination of B$_6$ vitamers. Brain extracts to be assayed were prepared by homogenizing about 400 mg of brain with 9 vol. of cold 1 N perchloric acid, centrifuging, reextracting the residue twice with 3 vol. of cold 0.2 N perchloric acid, and neutralizing the combined extracts with 5 N KOH to pH about 6.0.

Seven milliliters of the tissue extract thus obtained was applied to a cationic exchange column (Amberlite CG-120, 1 × 3.5 cm) and the elution was performed according to the procedure of Loo and Badger (17). B$_6$ vitamers and its derivatives were well resolved and come off the column as follows; PLP and PLP-thiazolidine, the extract+10 ml of water; PMP, 25 ml of 0.1 M acetate buffer, pH 4.0 after 30 ml of 0.01 M acetate buffer, pH 4.0; PL-thiazolidine, 20 ml of 0.1 M acetate buffer, pH 5.0; PL, 25 ml of 0.1 M Na phosphate buffer, pH 6.0; PN, 20 ml of 0.1 M Na phosphate buffer, pH 6.5; PM, 25 ml of 0.1 M Na phosphate buffer, pH 8.5.

The assay of each B$_6$ vitamer with Saccharomyces carlsbergensis 4228 was performed by the procedure previously reported (18). Since PL-thiazolidine was also active for the yeast, the fraction containing both PLP and PLP-thiazolidine was hydrolyzed by acid phosphatase to PL and PL-thiazolidine (18), respectively, and the hydrolyzed products were separated again with the same column, and then each one was assayed with the yeast.

RESULTS AND DISCUSSION

Effect of DL-PeA on the contents of B$_6$ vitamers in brain

The effect of convulsant dose of DL-PeA on the contents of B$_6$ vitamers was
### Table 1. Effect of DL-PeA and PN on distribution of B₆ vitamers in mouse brain.

| Treatment           | Dose (mg/kg) | Time of decapitation (min) | PLP        | PMP        | PL (ng/g wet wt.) | PN (ng/g wet wt.) | PM (ng/g wet wt.) | PLP-thiaz. |
|---------------------|--------------|----------------------------|------------|------------|-------------------|-------------------|-------------------|------------|
| Control             | (5)          |                            |            |            |                   |                   |                   |            |
| DL-PeA              | (5)          | 500                        | At seizures (114±7) | 1,480±81   | 1,695±23          | 120±20            | 6±1               | 8±1        |
| DL-PeA+PN           | (5)          | 500±300                    | 114         | 1,379±69   | 1,798±24          | 2,052±179         | 707±105           | 2,149±321  |
| Control             | (5)          |                            |            |            |                   |                   |                   |            |
| DL-PeA              | (5)          | 500                        | At seizures (108±8) | 1,499±51   | 2,469±45          | 95±8              | 3±1               | 17±2       |
| Control             | (4)          | 450                        | At seizures (118±9) | 1,823±90   | 2,487±49          | 88±7              | 4±1               | 15±2       |
| Control             | (5)          |                            |            |            |                   |                   |                   |            |
| PN                  | (5)          | 300                        | 115         | 1,902±79   | 2,050±58          | 52±11             | 7±1               | 30±2       |
|                     |              |                            |            |            |                   |                   |                   |            |

\(a\) Number of determinations in parentheses.

\(b\) Each value is the mean±S.E.M.

### Table 2. Effect of D-PeA on distribution of B₆ vitamers in mouse brain.

| Treatment | Dose (mg/kg) | Time of decapitation (min) | PLP        | PMP        | PL (ng/g wet wt.) | PN (ng/g wet wt.) | PM (ng/g wet wt.) | PLP-thiaz. |
|-----------|--------------|----------------------------|------------|------------|-------------------|-------------------|-------------------|------------|
| Control   | (5)          |                            |            |            |                   |                   |                   |            |
| D-PeA     | (5)          | 500                        | 108        | 1,499±51   | 2,469±45          | 95±8              | 3±1               | 17±2       |
| Control   | (8)          | 500                        | 120        | 1,716±52   | 2,082±60          | 97±13             | 5±1               | 13±3       |
| D-PeA     | (8)          |                            |            |            |                   |                   |                   |            |
| Control   | (4)          | 250                        | 120        | 1,667±39   | 2,473±114         | 90±8              | 4±1               | 20±4       |
| D-PeA     | (4)          |                            |            |            |                   |                   |                   |            |

\(a\) Number of determinations in parentheses.

\(b\) Each value is the mean±S.E.M.
determined at the onset of seizures (Table 1). DL-PeA administration induced a significant decrease of PLP concentration and tended to decrease that of PL. In contrast, the DL-PeA administration did not influence the PMP concentration and other B₉ vitamers. The fate of the PLP concentration, following the DL-PeA administration, is illustrated in Fig. 1. The diminution of the content of PLP which was caused by DL-PeA was already apparent immediately after the administration although the convulsions only occurred between 110 and 130 min later. This latency suggests that this fall in the PLP level in the brain may be causally related to the convulsant phenomenon. 

Since the DL-PeA-induced seizures are known to be prevented by the simultaneous administration of PN (II), experiments with this B₉ vitamer were included in the present investigation. The administration of PN along with DL-PeA prevented convulsions induced in mice by DL-PeA and lessened the concomitant decrease of PLP concentration without altering PMP concentration. The PN administration gave also remarkable increases in the other two non-phosphorylated vitamers (Table 1). These results seem to suggest that there are rapid inter-conversion reactions going on in the brain and that the recovery of PLP content is possibly the result of PLP formation through PL or PNP.

In spite of the marked diminution of PLP content by the DL-PeA administration, there was little amount of thiazolidine derivative of PLP in the mouse brain tested (Table 1). This may be due to elimination of the formed thiazolidine compound from the brain tissue.

An administration of PN alone gave remarkable increases in non-phosphorylated B₉ vitamers but did not significant changes in PLP and PMP (Table 1). This

![Fig. 1. Effect of intraperitonealy administered DL-PeA (450 mg/kg) on the PLP content of brain. Each point represents the mean of 5 determinations±S.E.M. The horizontal bracket indicates the time in the first convulsions±S.E.M. after dl-PeA treatment.](image-url)
result may represent a complete regulation of the phosphorylating mechanism, although there is no direct evidence to support this supposition.

**Effect of D-PeA on the contents of B₆ vitamers in brain**

The next studies were undertaken to test the effect of D-PeA on the distribution of B₆ vitamers in mouse brain. Since an injection of D-PeA could not cause attacks of convulsions, levels of B₆ vitamers after the D-PeA administration were determined at the time corresponding to a mean convulsion time when the same amount of DL-PeA was administered.

The effects of the D-PeA administration on the levels of B₆ vitamers in brain are reported in Table 2. The PLP content of brain was reduced to between 80 and 90% of the control level, and PLP- and PL-thiazolidine derivative were also produced in trace amounts. These results show that as compared with L-PeA, D-PeA causes a far slight diminution of PLP content which is accompanied by the production of PLP- and PL-thiazolidine compound.

Previous fact that L-PeA but not D-PeA exerts an antivitamin B₆ effect in animals may be interpreted by the difference in the potency reducing PLP content between L-PeA and D-PeA.

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