Gibberellin activity was noted twice a year, once in the growing season (May) and once in the dormant period (December) of the plant (table). The gibberellin activity in both healthy and diseased cuttings was high in May but decresaed greatly in December (to 5.53% in healthy and to 19.92% in diseased cuttings in terms of percentage average shoot length in cm). Thus while gibberellin activity in May was 22.18% less in diseased cuttings than in the healthy ones but in December the gibberellin activity was 13.63% higher in diseased than in healthy cuttings.

It appears from above that the higher quantity of gibberellins in diseased cuttings in December could be responsible for the break in bud dormancy of virus-infected stem cuttings of *E. pulcherrima* during the dormant season of the plant. This becomes all the more significant when these results are viewed in the context that, as already reported[5], the amounts of growth inhibitors (abscisic acid and phenols) present in diseased stem cuttings during the dormant period of the plant was less than in the healthy cuttings. It may also be mentioned that the pattern of changes of gibberellins in *E. pulcherrima* during the growing and dormant seasons was found to be the same as has already been reported widely[2,6,7]; that is, the amount of gibberellin decreases in plants with the onset of the dormant season. The degree of decrease in diseased plants (as shown above), however, is less so that the amount of gibberellins left behind in diseased stem cuttings are enough for the vegetative growth of the buds on them. The smaller amount of inhibitors in diseased cuttings also appears to be helpful in this connection.

Virus-infected *E. pulcherrima* seems to be the first virus-host combination in which studies of the break in bud dormancy, and estimations of endogenous growth regulators and growth inhibitors has been carried out. However, the role of gibberellins in the stunting of plants infected by mycoplasma-like organisms has been reported in only one study[13] while the physiological basis for this has been theoretically discussed[14]. The virus-host combination investigated by us may prove an ideal system in this connection.

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Changes of X-prolyl dipeptidyl-aminopeptidase activity in developing rat brain

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Summary. We found X-prolyl dipeptidyl-aminopeptidase activity in rat brain and examined the developmental changes at various ages. The total enzyme activity per brain increased until 4 weeks of age, and then decreased during maturation. Specific activity in young rat brain was higher than that in adult rat brain. The properties of the brain enzyme were different from those of pituitary and other tissues.

Protein turnover is highly active in the brain, and most proteins are in a dynamic state. Although changes of proteolytic enzymes in developing rat brain were reported by a few groups[1-3], the physiological role of the peptidases is not known. In our laboratory the physiological roles of X-prolyl dipeptidyl-aminopeptidase, which was discovered in rat liver and kidney by Hopsu-Havu and Glenner[4], have been studied in various tissues for the last several years[5-8]. Recently we found that X-prolyl dipeptidyl-aminopeptidase purified from human submaxillary gland[9] hydrolyzed N-terminal dipeptide Arg-Pro and subsequent dipeptide Lys-Pro from substance P[10]. Interestingly, the N-terminal tetrapeptide Arg-Pro-Lys-Pro-OH of substance P was shown to have almost the same effect as substance P on the neurite extension of neuroblastoma N-18 cells[11], and the N-terminal dipeptide Arg-Pro of substance P, which are cleaved by the enzyme, also had the same effect but neither arginine nor proline alone had the effect (Narumi and Maki, personal communication). We have also found X-prolyl dipeptidyl-aminopeptidase activity in human cerebrospinal fluid[8], suggesting the presence of the enzyme in the brain. The enzyme in the brain may release N-terminal dipeptide Arg-Pro from some brain peptides such as substance P and bradykinin. In this paper we report the presence, some properties and the changes during development of rat brain, of X-prolyl dipeptidyl-aminopeptidase activity.

Materials and methods. p-Nitroanilides (pNA) of Arg-Pro, Lys-Pro, Gly-Pro, Ala-Ala, Gly-Ala, Ala-Gly and Gly-Leu, and 7-(Gly-Pro)-4-methylcoumarinamide (Gly-Pro-MCA) were synthesized at Protein Research Foundation (Minoh, Osaka, Japan) as reported previously[7,12,13]. Sprague-Dawley rats were raised in our laboratory. At each age, the rat was killed by decapitation, and the brain was quickly removed, frozen and stored at −80 °C. The brain was homogenized in 3 vol. of 0.25 M sucrose. Enzyme activity of the brain homogenate at various ages was assayed with Gly-Pro-MCA as reported previously[13], except using Tris-maleate buffer, pH 7.0. The incubation mixture (total vol. 100 µl) contained 20 mM Tris-maleate buffer, pH 7.0, 0.5 mM Gly-Pro-MCA tosylate and enzyme. The incubation was done at 37 °C for 30 min and the reaction was stopped by adding 1.0 ml of 1 M sodium acetate buffer (pH 4.2). After centrifugation, the fluorescence intensity of 7-amino-4-methylcoumarin liberated by the enzyme reaction was measured at 460 nm with excitation at 380 nm. To

Table 1. Developmental changes of X-prolyl dipeptidyl-aminopeptidase activity in rat brain

Age	Brain weight (g)	Protein content (mg/g) of the brain	Activity nmoles/min per g of tissue	nmoles/min per brain	nmoles/min per mg of protein
4 days	0.344±0.021	56.3±2.2	692±21	238±20	12.33±0.69
1 week	0.454±0.044	62.7±1.1	707±20	300±24	11.28±0.23
2 weeks	0.980±0.004	70.4±1.2	878±16	860±15	12.47±0.24
3 weeks	1.330±0.026	84.2±0.9	942± 2	1253±25	11.20±0.11
4 weeks	1.414±0.041	90.4±1.6	920±15	1303±33	10.20±0.31
5 weeks	1.504±0.016	94.1±1.4	736±13	1108±12	7.83±0.20
7 weeks	1.540±0.029	98.0±1.7	637±14	983±22	6.50±0.09
20 weeks	1.882±0.117	99.1±1.2	463±11	867±42	4.67±0.09

Each value represents mean ± SEM, n = 5.

Table 2. Rates of hydrolysis of various dipeptide-p-nitroanilides by rat brain and pituitary enzymes partially purified by Sephadex G-200 gel filtration

Substrate	Relative activity (%) Brain	Pituitary	Brain/ pituitary
Gly-Pro-pNA	100[a]	100[b]	1
Lys-Pro-pNA	136	40	3.4
Arg-Pro-pNA	129	53	2.4
Ala-Ala-pNA	76	168	0.45
Gly-Ala-pNA	70	31	2.3
Ala-Gly-pNA	3.7	6.0	0.62
Gly-Leu-pNA	3.2	3.1	1.0

Rat brain at 3 weeks of age was used. Activities were measured at pH 5.8 in 90 mM acetate buffer and at a substrate concentration of 1.4 mM. The values are the mean of duplicate experiments. [a]43.2 moles/min/mg protein, [b]57.5 nmol/min/mg protein.

see the substrate specificity for various dipeptide-p-nitroanilides, brain and pituitary dipeptidyl-aminopeptidases were partially purified. 3-week-old rat brain (1.4 g) was homogenized in 3 vol. of 0.25 M sucrose. The homogenate was centrifuged at 100,000×g for 60 min, 1.3 ml of the supernatant fluid was applied to a Sephadex G-200 column (1.6×58 cm) equilibrated with 5 mM Tris-HCl (pH 7.4), and active fractions were pooled. 12 pieces of 3-week-old rat pituitary glands (12.5 mg) were homogenized in 1.25 ml of 0.25 M sucrose. After centrifugation of the homogenate, the supernatant was separated (1.25 ml) and applied to the same Sephadex column, and active fractions were pooled.

Results and discussion. X-Prolyl dipeptidyl-aminopeptidase activity was found in rat brain with Gly-Pro-MCA as a substrate. Total dipeptidyl-aminopeptidase activity of the rat brain at 3 and 4 weeks of ages was the highest in all the ages studied, and the enzyme activity per g of tissue was also high at these young ages (table 1). Specific activity per mg protein was almost similar during the period between 4 days and 4 weeks of ages, but decreased with maturation after 4 weeks of age. The specific activity of the enzyme in young rat brain was about 2.5 times higher than that in adult one. These developmental changes are similar to those of the myelination in rat brain. These results suggest that X-prolyl dipeptidyl-aminopeptidase in rat brain may be related to the development of the nervous system.

Analytical recovery studies were done to show that the changes of X-prolyl dipeptidyl-aminopeptidase in developing rat brain does not depend on the amount of endogenous inhibitor(s) in rat brain. When the rat brain enzyme partially purified by Sephadex G-200 gel filtration was added to the homogenate of rat brain at all ages, 95–100% of the activity could be accounted for, implying that these changes of X-prolyl dipeptidyl-aminopeptidase activity are not due to the amount of endogeneous inhibitor in rat brain.

The properties of the enzyme in rat brain were examined after partial separation by Sephadex G-200 column chromatography in comparison with the rat pituitary enzyme. The optimum pH and molecular weight for rat brain dipeptidyl-aminopeptidase were similar to those of the pituitary enzyme; 5.8 and 130,000. However, as shown in table 2, the rate of hydrolysis of Lys-Pro-pNA or Arg-Pro-pNA by rat brain dipeptidyl-aminopeptidase was about 1.3 fold higher than that of Gly-Pro-pNA, while the rate of hydrolysis of Lys-Pro-pNA or Arg-Pro-pNA by the pituitary dipeptidyl-aminopeptidase was about half that of Gly-Pro-pNA. The difference in the substrate specificity between the 2 enzymes suggests that the physiological role of rat brain dipeptidyl-aminopeptidase may be different from that of the pituitary enzyme. The properties of the brain enzyme were also different from those of the enzyme in peripheral tissues such as the submaxillary gland[9]. Since Arg-Pro has almost the same effect as substance P on the neurite extension of neuroblastoma (Narumi and Maki, personal communication), rat brain dipeptidyl-aminopeptidase could be related to the development of neurones by forming the dipeptide.

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