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Analysis of tryptophan, tyrosine and related dipeptides in mouse brain by isocratic high-performance liquid chromatography with switchable wavelength fluorescence detection*

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Abstract: Tryptophan (Trp) and tyrosine (Tyr) are pharmacologically active compounds which, after administration of adequate doses, are increased in level in the brain, and stimulate neurotransmitter synthesis. Trp and Tyr containing dipeptides were tested as possible substitutes with regard to the effect on precursor level in the brain. Glycyl-tryptophan, alanyl-tryptophan and glycyl-tyrosine were intravenously applied to young female mice and the brain levels of dipeptides, Trp and Tyr measured 30 min after application. Neurotransmitter precursor levels in the brain increased similarly in all cases. The results suggest that the dipeptides are as effective as the single amino acids and may be superior because of their better solubility.

Keywords: *Brain; dipeptides; HPLC; tryptophan; tyrosine.*

Introduction

The amino acids tryptophan (Trp) and tyrosine (Tyr) are neurotransmitters and pharmacologically active compounds [1–3]. In consequence they are recommended as therapeutic agents in various clinical situations [4–7].

Trp and Tyr pass the blood brain barrier without difficulty and their brain level is directly correlated to the blood level [1–3]. Thus brain neurotransmitter precursor levels can be influenced by the ingestion of food or by the application of the single amino acid [3]. From animal experiments it is known that an elevation of the Trp level in the brain can be achieved by doses of 25 mg kg^{-1} either by the oral or by the intravenous route [13]. For tyrosine much higher doses than for tryptophan have to be applied in order to increase the brain Tyr level to a measurable degree [14, 15].

The use of both amino acids is limited by their poor stability and solubility and for this reason, for example, tyrosine is either omitted from many so called complete amino acid mixtures or substituted by the xenocompound *N*-acetyl-tyrosine [8, 9]. Recently glycyl-

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tyrosine [10] and alanyl-tyrosine [11] have been tested during total parenteral nutrition of the rat.

Dipeptides containing Trp or Tyr (for example glycyl or alanyl compounds) are more soluble in water than the single amino acids and should be superior in pharmacological applications, under the premise that the subject handles them in the same manner as the single amino acids. Recently we have been able to show that the bioavailability of Trp in the peripheral blood after intragastric application in rats is the same both when Trp is given alone or as a dipeptide with alanine or glycine [12]. This suggests a very rapid splitting of the dipeptides in the intestine.

The question arose whether the dipeptides could also be given by the venous route and whether it is possible to elevate brain neurotransmitter precursors to the same degree as with the single amino acids. Glycyl-tyrosine (Gly-Tyr), alanyl-tryptophan (Ala-Trp) and glycyl-tryptophan (Gly-Trp) were studied in a mouse model.

Materials and Methods

The dipeptides Gly-Tyr, Gly-Trp and Ala-Trp were obtained from Serva (Heidelberg, FRG), all other chemicals were from Merck (Darmstadt, FRG).

HPLC was performed with a liquid chromatograph series 10 and a spectrofluorimeter type LS-5 detector (Perkin–Elmer, Überlingen, FRG). The equipment contained a wavelength-scan facility and a wavelength programmer for the optimal individual detection of Trp, Tyr and the different dipeptides at their respective optimum wavelengths. In its simplest form a chromatographic run was monitored at 276/313 nm for Tyr for 8 min and switched thereafter to 276/350 nm for Trp. The flow rate was 1.0 ml min^{-1} and the peak height was registered on a 561 recorder (Perkin–Elmer, Überlingen, FRG). The stationary phase was Nucleosil C_{18} 5 μm (Macherey–Nagel, Düren, FRG) in a V4A steel column (250 × 4.6 mm i.d.). The mobile phase consisted of 0.05 M citric acid/0.05 M sodium acetate/17% methanol, pH 4.3, prepared with double distilled water filtered through a Millipore® Q cartridge purification system. After preparation the buffer was again filtered through Millipore® 0.45 μm membranes.

Three experiments involving 67 young female mice were performed (NMRI, Versuchstierzucht, Hannover, FRG). The animals received the amino acids or peptides through the tail vein. Tyr, Trp or the peptides were dissolved in equimolar concentrations in 0.9% NaCl and 100 μl of the solution was applied to each mouse. The animals were decapitated 30 min after the application and the brains frozen in liquid nitrogen as quickly as possible (less than 1 min). Storage of tissue until the determination of free Tyr or Trp concentration in the brain was less than 7 days. The brains were homogenized in 0.4 M perchloric acid (2 ml for each 50 mg tissue) and the mixture centrifuged at 20,000 **g** for 15 min. The clear supernatant (50 μl) was injected into the chromatographic system.

Statistical calculations were performed with the U-test from Mann–Whitney [16].

Results

A full chromatogram from a mouse brain extract spiked with the dipeptides is shown in Fig. 1. Fluorimetric detection of the single compounds was performed at their excitation/emission wavelength maxima: Tyr 276 nm/313 nm; Gly-Tyr 270 nm/307 nm; Trp 276 nm/350 nm; Gly-Trp 278 nm/360 nm; Ala-Trp 275 nm/360 nm.

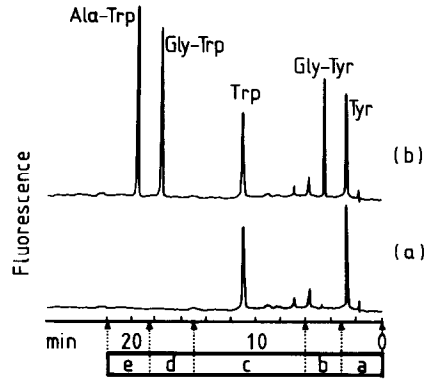

Figure 1
Elution pattern of isocratic reversed-phase HPLC of mouse brain extracts with switchable wavelength fluorescence detection. The extracts were run without (a) and with (b) added dipeptides (10 nmol ml^{-1}). Wavelength programme: a, 0–2.7 min at 276 nm/313 nm; b, 2.7–6.0 min at 270 nm/307 nm; c, 6.0–15.0 min at 276 nm/350 nm; d, 15.0–18.2 min at 275 nm/360 nm; e, 18.2–22.0 min at 278 nm/360 nm.

Table 1
Data on the technology of the HPLC dipeptide assay. Indicated is the mean ± standard deviation

	Retention time (min)	Detection limits* (pmol)	Intraday (%)	RSD† interday (%)	Recovery‡ (%)
Tyr	2.3 ± 0.4	19.8 ± 4.1	1.49	2.41	103.5 ± 9.8
Gly-Tyr	3.1 ± 0.4	52.4 ± 6.2	0.68	2.79	103.2 ± 8.8
Trp	10.8 ± 1.2	6.6 ± 0.9	2.68	3.94	103.5 ± 11.0
Gly-Trp	17.6 ± 1.1	20.2 ± 2.9	1.29	3.90	95.9 ± 11.1
Ala-Trp	19.5 ± 1.8	38.9 ± 5.1	2.74	4.88	105.3 ± 4.9

*Quantity of standard injected giving a signal-to-noise ratio of 2 ($N = 3$).
†Relative standard deviation for a standard mixture (0.6–1.7 nmol ml^{-1}), $N = 8$–17 determinations.
‡Mean ± standard deviations from 3 pooled brain extracts with or without the addition of standard compounds (0.6–1.7 nmol ml^{-1}).

Data on the technology of the applied dipeptide assay are summarized in Table 1. A linear relationship between concentration and peak height (cm) was observed over the concentration range studied (0–300 pmol/50 µl). The equations for the calibration curves were $y = 0.021\ x - 0.0018$ for Tyr ($r = 0.9999$); $y = 0.024\ x - 0.166$ for Gly-Try ($r = 0.9946$); $y = 0.020\ x + 0.125$ for Trp ($r = 0.9908$); $y = 0.0083\ x - 0.072$ for Ala-Trp ($r = 0.9994$); $y = 0.0071\ x + 0.048$ for Gly-Trp ($r = 0.9967$); $N = 6$ for each equation.

In none of the experiments with mice could an intact dipeptide molecule be detected either in blood or brain tissue.

In two experiments either Gly-Trp or Ala-Trp were given and the results compared with Trp alone, 0.9% NaCl being given to the control group. It can be seen from Figs 2 and 3 that the brain level of Trp was significantly ($P \leq 0.01$) elevated 30 min after the intravenous infusion of Trp, Gly-Trp and Ala-Trp respectively. There is no statistical indication for a difference between the two results for the Trp-containing peptides or between either of the dipeptides and the single amino acid.

In the third experiment Gly-Tyr was given in three doses of 30, 60 and 120 µmol kg^{-1}, but Tyr was only given at the two lower dosage levels because at the highest dose Tyr precipitated out of solution.

At the low dose of 30 µmol kg^{-1} no effect on the brain Tyr level was observed (Fig. 4); 60 µmol kg^{-1} caused a slight but statistically not significant elevation (versus NaCl-controls). At 120 µmol kg^{-1} a statistically significant ($P \leq 0.01$) increase of brain Tyr content to about twice the normal level in mouse brain tissue was observed.

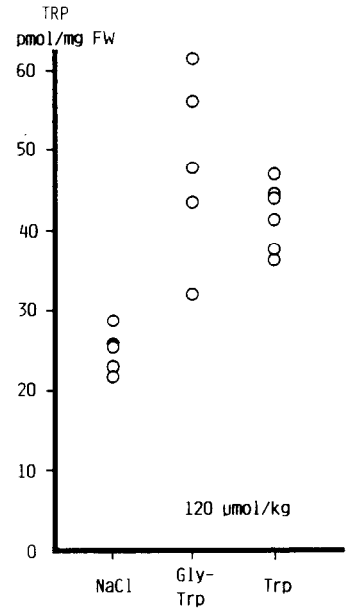

Figure 2
Increase of brain Trp after intravenous administration of 120 µmol kg^{-1} glycyl-tryptophan to young female mice compared with an equimolar amount of i.v. administered tryptophan and 0.9% NaCl. Each circle represents one animal ($N = 16$).

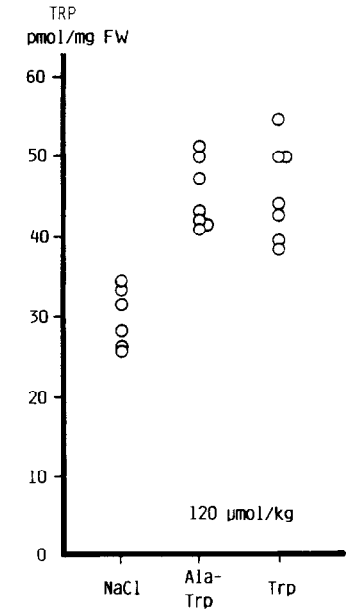

Figure 3
Increase of brain Trp after intravenous administration of 120 µmol kg^{-1} alanyl-tryptophan to young female mice compared with an equimolar amount of i.v. administered tryptophan and 0.9% NaCl. Each circle represents one animal ($N = 20$).

Discussion

The aim of this study was to test whether Trp and Tyr containing dipeptides are principle substitutes for the poorly soluble and unstable amino acids Trp and Tyr. The results show that brain precursor levels are elevated to a similar degree after amino acid or dipeptide administration.

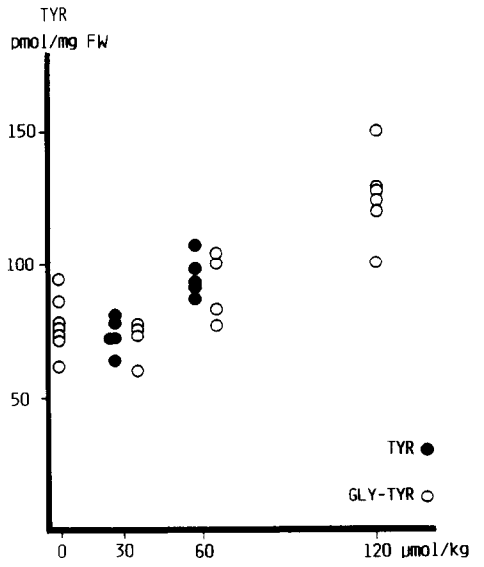

Figure 4
Increase of brain tyrosine after intravenous administration of different doses of glycyl-tyrosine (abscissa) to young female mice compared with equimolar amounts of i.v. administered tyrosine and a control group with 0.9% NaCl. Each circle represents one animal (N = 31). At 120 µmol kg^{-1} tyrosine could not be administered due to its poor solubility.

The experiments with mouse brain do not give any information on the fate of the dipeptides within the blood, but it is reasonable to assume that they are rapidly split into monomeric compounds. This assumption is consistent with observations of a short half-life and rapid hydrolysis of other dipeptides in rat plasma [17, 18].

The studies with Gly-Tyr revealed the possible advantage in the use of the dipeptides. Brain Tyr levels were increased two-fold by a dose of 120 µmol kg^{-1}. Recently Ablett *et al.* [14] studied the levels of free Tyr in different rat tissues after Tyr application. A Tyr suspension was given intraperitoneally in order to bring enough Tyr into the body. With 200 mg kg^{-1} a ten-fold dose was applied but only a three-fold elevation of brain Tyr level was achieved in comparison to the effect of Gly-Tyr.

This difference might be caused by a number of factors e.g. volume administered, rate of dissolution of Tyr from suspension, rate of absorption from the suspension etc. All of these factors are somehow related to the poor solubility of Tyr and might be overcome by the administration of Gly-Tyr.

It should be noted that the rat study [12] and the present experiments with mice are the first to indicate the pharmacological applicability of dipeptides containing Trp and Tyr. Further studies have to be performed to take advantage of these promising compounds and to check carefully their pharmacologic efficacy.

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