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Note

High-performance liquid chromatographic determination of (Me)Arg–Lys–Pro–Trp–*tert.*-Leu–Leu in plasma

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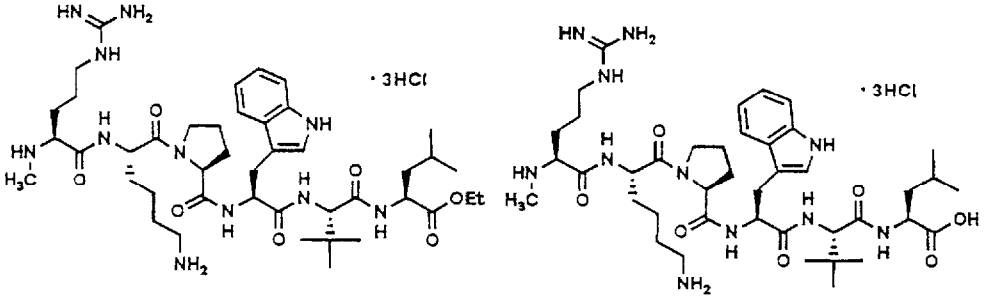
(Me)Arg–Lys–Pro–Trp–*tert*–Leu–Leu–OEt (I, Fig. 1) (Me = methyl; Et = ethyl) is a synthetic hexapeptide with neurotensin (NT) activity [1]. NT is distributed widely in the mammalian central nervous system, especially in the hypothalamus, amygdala, nucleus accumbens and ventral tegmental area [2]. It has been suggested that NT is closely associated with the dopaminergic neurons, particularly in the mesolimbic system, and that one of the physiological roles of NT is the modulation of dopaminergic neurons [3–5]. Therefore, compound I can be used as a new type of psychotropic drug [1].

This paper describes a simple high-performance liquid chromatographic (HPLC) procedure for the determination of I and of (Me)Arg–Lys–Pro–Trp–*tert*–Leu–Leu (II, Fig. 1) in rat plasma, and the preliminary pharmacokinetics in rats after single intravenous administrations of I and II.



Compound I Compound II

Fig 1 Structures of compounds I and II.

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EXPERIMENTAL

Materials

The solvents used were all of HPLC grade (Wako, Osaka, Japan) and all other reagents were of analytical-reagent grade. Compounds I and II were synthesized by conventional methods in solution using the mixed anhydride coupling procedure [1]. They were deprotected and purified by low-pressure LC on carboxymethylcellulose followed by HPLC. The structures of I and II were confirmed by mass and NMR spectrometry and by amino acid analysis. The molecular masses of I and II were 963.49 and 935.44, respectively.

Apparatus and chromatographic conditions

The HPLC system consisted of a Model LC-6A pump, equipped with a Model SCL-6B system controller, a Model RF-530 fluorescence monitor, a Model CTO-6A column oven, a Model C-R4AX Chromatopac and a Model SIL-6B autoinjector, all from Shimadzu (Kyoto, Japan) The chromatographic column was a YMC Pack AM-312 ODS (150 mm × 6 mm I.D., particle diameter 5 μm) obtained from Yamamura Chemical Lab. (Kyoto, Japan). The mobile phase for elution of I and II was acetonitrile–water–perchloric acid (60%) (316.684:1.5 and 260·740:1.6, v/v, respectively) of pH 2 0 and 1.8, respectively. The flow-rate was 1.0 ml/min. The excitation and emission wavelengths were 280 and 350 nm, respectively. The injection volume was 50 μl. The separations were performed at 40°C.

Standard solution

A standard solution of II was prepared at 1 mg/ml in 0.9% saline (pH 6.3) and stored at 4°C. A solution of ethyl *p*-hydroxybenzoate as an internal standard (I.S.) was prepared at 20 μg/ml in ethanol and stored at room temperature. A calibration graph was obtained by adding known amounts of II to rat plasma cooled in an ice-bath. These plasma standards were treated as described below.

Assay procedures

A 150-μl volume of methanol containing 0.2% perchloric acid and 50 μl of I.S. was added to 100 μl of plasma cooled in an ice-bath. The mixture was stirred on a vortex mixer for 1 min and centrifuged at 1800 *g* for 10 min at 4°C; 50 μl of the supernatant were injected into the chromatograph.

Calculations

For the determination of II in plasma, the peak-area ratios of II to the I.S. were compared with those for the plasma standard.

Animal study

Male Wistar rats, weighing 250–300 g and fasted overnight, were treated in-

travenously with I and II at a dosage of 1 mg/kg. Componds I and II were dissolved at 1 mg/ml in the saline. Blood samples were drawn at different times (5, 10, 20, 30, 60, 90 and 120 min) after administration. The blood samples were cooled to 0–5°C in an ice-bath, and the plasma samples were separated by centrifugation at 2050 *g* for 10 min at 4°C. The plasma levels of II were determined according to the above-mentioned assay procedure.

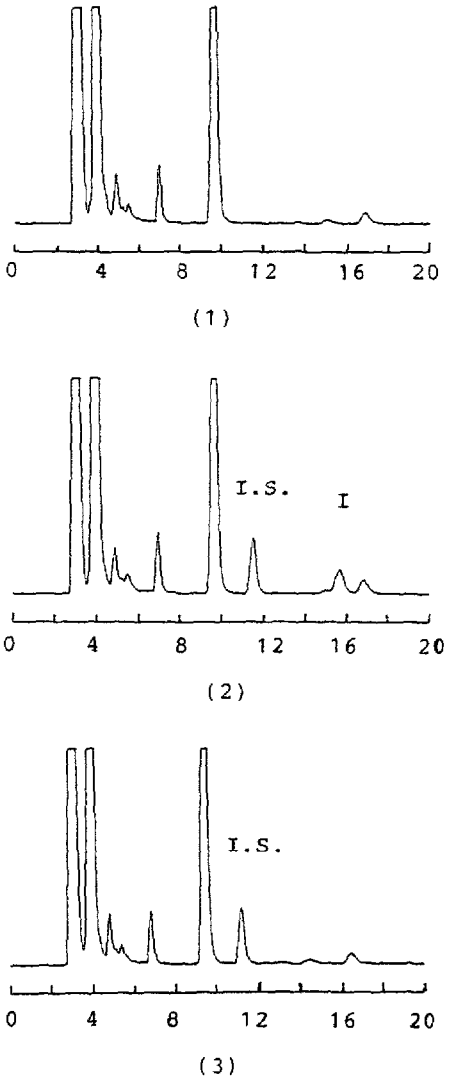

Fig. 2 HPLC of rat plasma containing I and I S (1) Drug-free plasma, (2) plasma spiked with I at 1 μg/ml; (3) plasma of rats treated with I. Chromatographic conditions as described under Experimental A 100-μl sample of plasma was used for the assay

RESULTS AND DISCUSSION

Fig. 2 shows typical chromatograms of I obtained under the conditions described above. The retention times of I and the I.S. were 15.7 and 11.6 min, respectively. The two compounds were well separated. The chromatogram of drug-free plasma showed no detectable interference with endogenous substances in the plasma. However, after intravenous administration of I to the rat it could not be detected in the plasma.

Fig. 3 shows typical chromatograms of II in rat plasma under the conditions described above. The retention times were 11.6 min for II and 17.7 min for the I.S. The two compounds were well separated. The chromatogram of drug-free plasma showed no detectable interference with endogenous substances in the plasma. After intravenous administration of I and II to the rat, the plasma levels of II were measured. Compound I was undetectable, as stated above, and only II was detected. This phenomenon is due to the lability of I in rat plasma.

Compound II is efficiently extracted with methanol containing 0.2% perchlo-

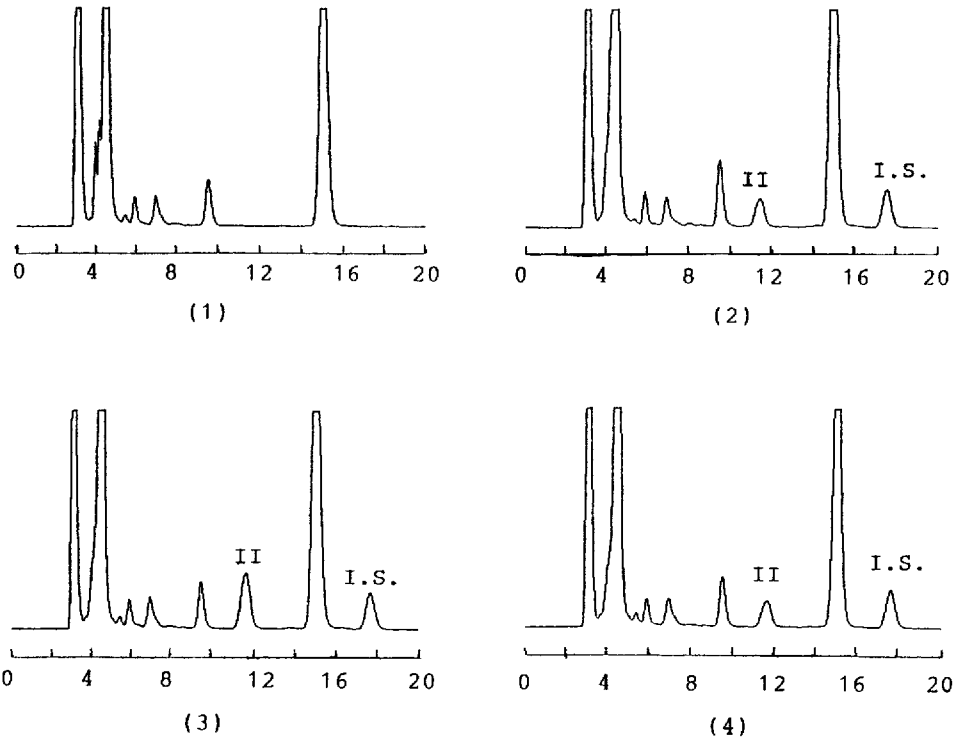

Fig. 3 HPLC of rat plasma containing II and I S (1) Drug-free plasma, (2) plasma spiked with II at 1 14 μg/ml, (3) plasma of rats treated with I, (4) plasma of rats treated with II. Chromatographic conditions as described under Experimental A 100-μl sample of plasma was used for the assay

TABLE I

ANALYSIS OF PLASMA SAMPLES CONTAINING KNOWN AMOUNTS OF COMPOUND II INTRA-ASSAY PARAMETERS

Theoretical concentration (μg/ml)	Peak-area ratio (mean ± S.D , $n=5$)	Concentration found (mean ± S D., $n=5$) (μg/ml)	Relative standard deviation (%)	Accuracy (mean) (%)
0 34	0 213 ± 0 012	0 353 ± 0 018	5 0	103 1
0 57	0 355 ± 0.008	0 559 ± 0 012	2 2	97.9
1 14	0.748 ± 0.016	1 126 ± 0 023	2.0	98.6
2.28	1.530 ± 0.054	2 255 ± 0 078	3.4	98.7
3 43	2.400 ± 0.038	3 507 ± 0 055	1.6	102.4
5 71	3.900 ± 0 074	5 675 ± 0 106	1.9	99 4

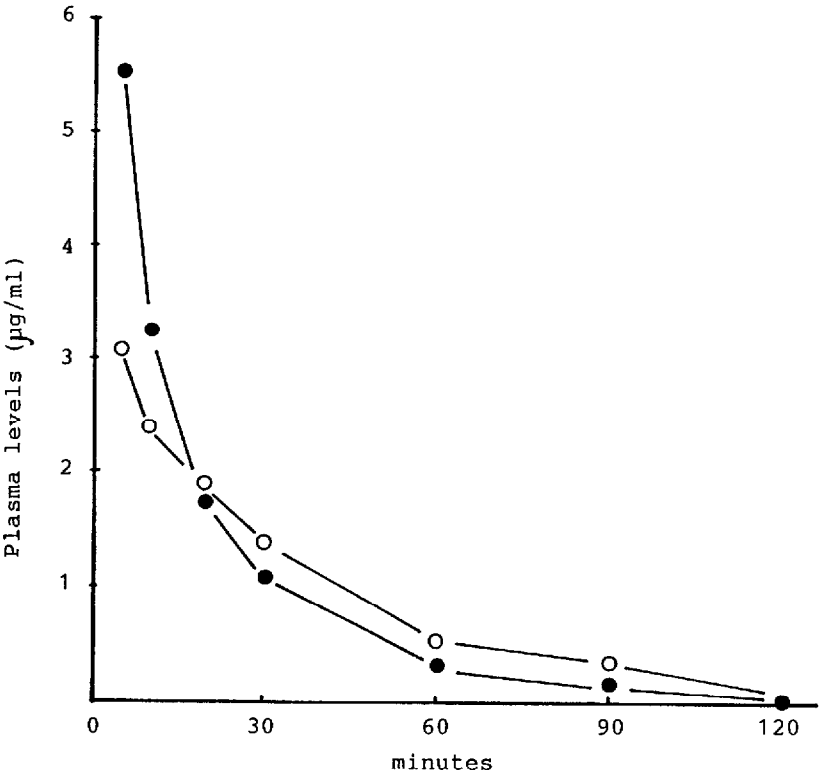

Fig 4 Plasma levels of II after intravenous administration of (●) I and (○) II at 1.0 mg/kg to rats

ric acid. Table I shows that the precision of the method is 5.0% or better, and that the accuracy ranges from 97.7 to 103.1%.

The calibration graph was linear from 0.3 to 5.7 μg/ml II in plasma. The relationship between the plasma concentration of II and the peak-area ratio is expressed by $y = 0.693x - 0.032$, where x is the concentration of II (μg/ml in plasma) and y is the peak-area ratio. The correlation coefficient (r) is 0.9998. The detection limit of II was calculated to be 40 ng/ml, with a signal-to-noise ratio of *ca.* 2:1.

The plasma levels of II in the rat after intravenous administration of I and II are given in Fig. 4. All values are the means for three animals. The plasma levels of II after intravenous administration of I and II can be described by a two-compartment model: the half-lives of elimination ($t_{1/2\beta}$) are 22.0 min for the intravenous administration of I and 80.6 min for that of II; the total clearance is 551 ml/h · kg for I and 500 ml/h · kg for II and the distribution volume is 231 ml/kg for I and 364 ml/kg for II.

In conclusion, the proposed method, requiring only a small volume of plasma (100 μl) and with rapid sample preparation, is simple, precise and accurate, and can be used in human and animal pharmacokinetic studies.

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