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Note

Simultaneous determination of tryptamine and its metabolites in mouse brain by high-performance liquid chromatography with fluorometric detection

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The indoleamines tryptamine (TRM) and 5-hydroxytryptamine (5-HT) are formed by decarboxylation from tryptophan (TRP) and 5-hydroxytryptophan, respectively. The presence of TRM in the mammalian brain has been demonstrated [1—3], although the level is very low compared with that of 5-HT.

It is well known that 5-HT is a neurotransmitter in the central nervous system (CNS) and that it plays an important role in physiological functions such as sleep or thermoregulation. Recently, there has been increasing interest in the physiological and pharmacological effects of TRM. It has been suggested that TRM may play a specific role in neurotransmission and neuromodulation in the CNS [4—7]. In behavioural studies, Dewhurst [8] has reported that TRM acts as an excitatory amine in young chickens and recently it has been proposed that TRM induces myoclonus in guinea pigs treated with the monoamine oxidase (MAO) inhibitor [9].

The involvement of TRM in neurological disorders has also been implied. An elevation of TRM excretion in the urine has been shown to occur in Parkinson's disease and schizophrenia [10, 11].

The turnover rates of trace amines are known to be rather rapid and a large increase in these amines has been observed in the rat brain after MAO inhibition [12, 13]. It has been reported that the half-life for TRM is less than 1 min which is extremely rapid compared to 77 min for 5-HT [14]. TRM is immediately metabolized to indoleacetaldehyde by MAO and is consequently converted to indoleacetic acid (IAA) or tryptophol (TOL).

The occurrence of IAA in the rat brain has been reported [15, 16]. IAA is a major metabolite of TRM and Young et al. [17] suggested that the concentra-

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tion of IAA in the cerebrospinal fluid can be used as an index of TRM turnover. Recently, we demonstrated that TOL is present in the mouse brain as a TRM metabolite [18]. In addition, TOL has been shown to have pharmacological properties [19, 20], and an increase in the IAA level has been observed in several neurological disorders [21, 22]. It is suggested that the measurement of indoles in the TRM pathway may be significant in neurochemical studies.

Until now, the determination of TRM has been performed using fluorometry or gas chromatography—mass spectrometry [1, 23]. These techniques require complicated extraction procedures. Recently, high-performance liquid chromatography (HPLC) has been developed and applied for the determination of biological samples. We have previously reported the simultaneous determination of TRP and its metabolites by HPLC with fluorometric detection [24].

In this paper, we show a simple, sensitive and simultaneous method for the determination of indoles in the TRM pathway, i.e. TRM, IAA and TOL, in mouse brain by HPLC with fluorometric detection.

MATERIALS AND METHODS

TRP and IAA were purchased from Nakarai (Kyoto, Japan). TOL was obtained from Sigma (St. Louis, MO, U.S.A.) and TRM from Wako (Osaka, Japan). The other chemicals were reagent grade. The standard solution was prepared in 0.1 *M* perchloric acid.

Male ddY mice weighing 20—25 g were obtained from Shizuoka Laboratory Animals (Shizuoka, Japan). Mice were killed by decapitation and brains were rapidly removed and weighed. TRM was dissolved in saline and injected intraperitoneally at 50 mg/kg. The mice treated with TRM were killed 2 min after the injection. The brains were stored at −40° C until analysis.

Each brain was extracted according to the method detailed in our previous report [24]. The brain was homogenized in 4 ml of 0.1 *M* perchloric acid containing 0.02% ascorbic acid. The homogenate was centrifuged at 15,000 *g* for 10 min at 0° C. The supernatant was transferred to a sample tube and the residue was resuspended and centrifuged again. The supernatants were pooled. After filtering through a 0.45-μm filter, 20 μl of supernatant were injected directly into the HPLC system.

The chromatography was performed with a Shimadzu (Kyoto, Japan) LC-3A liquid chromatograph. A Zorbax C_8 reversed-phase column (10 μm particle size, 250 mm × 4.6 mm I.D.) was used for the separation of the indoles. The mobile phase consisted of 50 m*M* acetate buffer (pH 5.0) containing 35% methanol. The fluorometric detection was performed with an RF-530 spectrophotofluorometer (Shimadzu, Japan). The excitation and emission wavelengths were set at 280 and 350 nm, respectively. The flow-rate was 0.7 ml/min. The column temperature was maintained at 24 ± 1° C during the analysis.

The concentrations of indoles were determined from peak heights compared with external standards. The retention times and peak heights were obtained using a Chromatopac C-R1B data processor (Shimadzu).

RESULTS AND DISCUSSION

A large number of methods for determining indole compounds, including gas chromatography—mass spectrometry, fluorometry and HPLC, have been described [25]. HPLC with fluorometric detection offers high specificity for the measurement of indoles using their native fluorescence. Recently, we also reported a sensitive method for the determination of TRP and its metabolites using HPLC with fluorometric detection [24]. However, there are few research reports about the sensitive determination of TRM and its metabolites [1, 15]. We developed the assay and applied it for the sensitive and simultaneous determination of TRM and its metabolites by HPLC with fluorometric detection.

Fig. 1 shows the chromatogram of the standard solution. TRP, TRM, IAA and TOL were clearly separated by the C_8 reversed-phase column. In addition to the three indoles in the TRM pathway, TRP, the precursor amino acid of the indoleamines, was eluted simultaneously.

The retention times and detection limits of the indoles are summarized in Table I. The limit of sensitivity is low, in the range 10—20 pg. The standard curves of peak height for the indoles were linear over the range 0.1—50 ng.

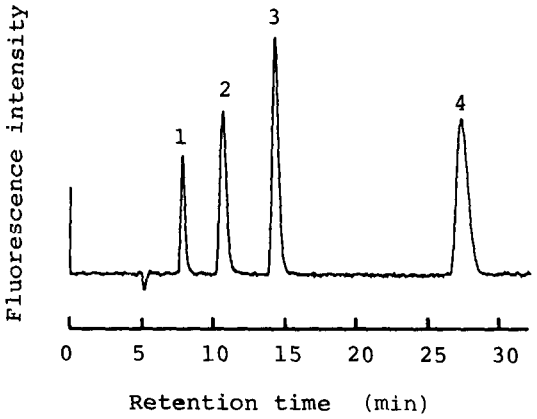

Fig. 1. Chromatogram of a standard mixture of 1 ng of each indole. Peaks: 1 = TRP; 2 = TRM; 3 = IAA; 4 = TOL. For chromatographic conditions, see text.

TABLE I

RETENTION TIMES AND DETECTION LIMITS OF INDOLES

For chromatographic conditions, see text.

Compound	Retention time (min)	Detection limit (pg)*
Tryptophan	8.10	20
Tryptamine	10.92	15
Indoleacetic acid	14.57	10
Tryptophol	28.02	15

*Injected quantity giving a signal-to-noise ratio of 2.0.

Reproducibility was examined by repeated injections of 1 ng standard solution. Standards were determined with the coefficients of variation in peak heights and retention times being 0.9—3.6%.

In Fig. 2A a typical chromatogram of a normal mouse brain sample is shown. TRP was monitored at 8.1 min. The mean concentration of TRP was 3497 ± 42 ng/g (mean ± S.E., n = 7), which agrees with previous reports [26, 27].

Fig. 2B shows the chromatogram of brain of a TRM-treated mouse. TRM was injected at 50 mg/kg and the mouse was killed 2 min after the treatment. TRP, TRM, IAA and TOL were detected and the concentrations for the sample shown in Fig. 2B were 4568 ng/g TRP, 325.3 ng/g TRM, 779.4 ng/g IAA and 66.6 ng/g TOL.

The recoveries for the brain (mean ± S.E.) were: TRP 90 ± 3.4%; TRM 87.1 ± 0.9%; IAA 82 ± 2.2%; TOL 86 ± 1.3%.

Identification of peaks was made by changing the percentage of methanol and pH in the mobile phase. The sample peaks were coeluted with the standards.

The half-life of TRM is known to be very short and it has been thought that TRM is mainly metabolized to IAA. Recently, we reported the occurrence and formation of TOL as a metabolite of TRM [18]. In this study, we have further confirmed that both IAA and TOL are formed from TRM.

It has been suggested that TRM is a neuroactive amine and that it possibly plays a role as a neurotransmitter or neuromodulator in the CNS [4—7]. In

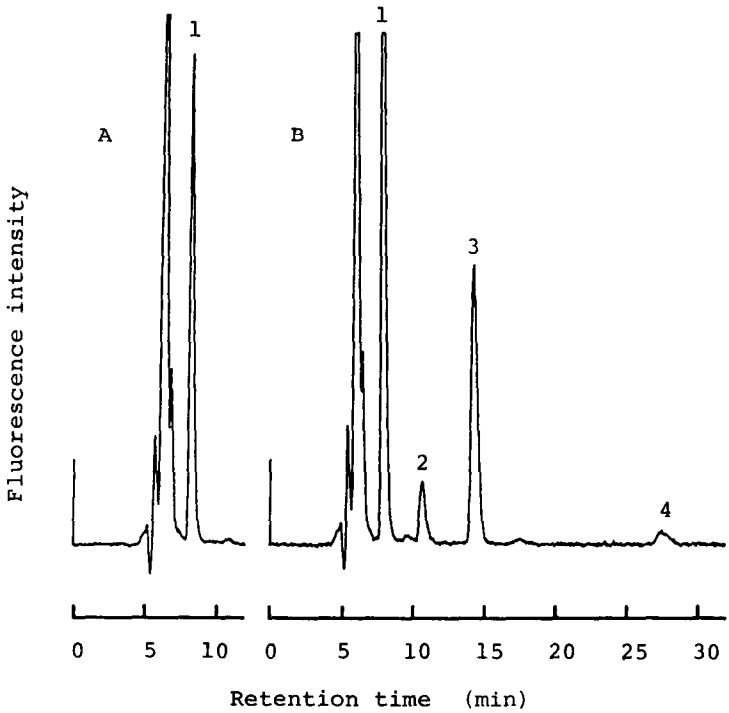

Fig. 2. Chromatograms of mouse brain samples: (A) normal mouse brain; (B) brain from mouse treated with 50 mg/kg TRM. Peaks: 1 = TRP; 2 = TRM; 3 = IAA; 4 = TOL. For chromatographic conditions, see text.

clinical studies, too, there are some indications that TRM may act in this way in neurological disorders [10, 11]. It has also been suggested that, besides TRM itself, the metabolites of TRM, IAA and TOL are involved in neurochemical functions [19—22]. Therefore, it would seem useful for neurochemical studies to elucidate the TRM metabolism.

In conclusion, the described method is quite simple, accurate and sensitive. This is the first report allowing the simultaneous determination of indoles in the TRM pathway. Our assay will be useful for follow-up investigations of TRM metabolism and offers great advantages to pharmacological and neurochemical studies of TRM.

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