Biochemical Pharmacology, Vol. 32, No. 9, pp. 1507–1510, 1983.
Printed in Great Britain.

0006-2952/83/091507-04 $03.00/0

OCCURRENCE OF 6-HYDROXY-1-METHYL-1,2,3,4-TETRAHYDRO-β-CARBOLINE IN TISSUES AND BODY FLUIDS OF RAT

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(*Received* 3 *September* 1982; *accepted* 13 *December* 1982)

Abstract—A capillary column gas chromatographic–mass spectrometric method was used to identify and quantitate 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline (6OMTHBC) in rat. The excretion rate in urine was 0.73 ± 0.20 nmoles per 24 hr and in faeces 0.18 ± 0.03 nmoles per 24 hr. In urine, about 90% of the 6OMTHBC was in a conjugated form, whereas in faeces most (~ 75%) of the 6OMTHBC was in a free form. The compound was detectable in liver (11.1 ± 3.6 pmoles/g), kidney (2.1 ± 0.9 pmoles/g) and plasma (0.52 ± 0.15 pmoles/ml), but not in brain (<0.3 pmoles/g). When 6OMTHBC was injected to rats, 75% of the injected amount was excreted in urine during the first 10 hr. The plasma level of 6OMTHBC declined with a half-life of 1.5 hr.

In recent years, several tetrahydro-β-carbolines (THBCs) have been reported to occur in tissues and body fluids of mammals [1]. The THBCs are physiologically active compounds which may function as neuromodulators or neurotransmitters [2]. However, their presence and significance has not yet been conclusively determined.

The formation of THBCs from endogenous indoleamines and acetaldehyde has been considered in the alcoholic syndrome [3]. Two compounds of this subclass of the THBCs have so far been studied. 1-Methyl-1,2,3,4-tetrahydro-β-carboline (1MTHBC) was detected in blood platelets, plasma and urine of healthy subjects after ethanol intake [4, 5]. Studies of 1MTHBC and 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-β-carboline (6OMTHBC) in this laboratory have revealed that these compounds are normal constituents of human urine [6, 7]. This stimulated us to perform the present study of the natural occurrence of 6OMTHBC in the rat.

MATERIALS AND METHODS

Chemicals and biological samples. 5-Benzyloxyindole, sulphatase type HI and 5-benzyloxytryptamine hydrochloride were obtained from Sigma Chemical Co. (St. Louis, MO); lithium aluminium deuteride from Fluka AG (Buchs, Switzerland); pentafluoropropionic anhydride (PFPA) from Reagenta (Uppsala, Sweden); and dichloromethane from Merck AG (Darmstadt, F.R.G.). Other chemicals used were of analytical purity.

5-Benzyloxy-($\alpha,\alpha,\beta,\beta$-$^2H_4$) tryptamine hydrochloride and 5-hydroxy-($\alpha,\alpha,\beta,\beta$-$^2H_4$) tryptamine picrate (5HT-$^2H_4$) were synthesized from 5-benzyloxyindole, according to Shaw *et al.* [8]. 6OMTHBC was synthesized from 5-benzyloxytryptamine hydrochloride according to Taborsky and Isaac [9]. Similarly, 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-(3,3,4,4-$^2H_4$)-β-carboline (6OMTHBC-$^2H_4$) was synthesized from 5-benzyloxy-($\alpha,\alpha,\beta,\beta$-$^2H_4$) tryptamine hydrochloride. The mass spectra of the dipentafluoropropionyl (PFP) derivatives of 6OMTHBC and 6OMTHBC-$^2H_4$ have been published previously [7].

Male rats (150 g) of the Sprague–Dawley strain Sweden). The rats were single-housed in metabolic cages and received food and water *ad libitum*. The urine was collected at 24 hr intervals in ice-cooled plastic vessels. At the end of a collection period, the faeces and urine were immediately put under storage (−70°).

The animals were killed by decapitation and the blood collected in Vacutainer tubes containing 1.5 ml of ACD-buffer (Becton, Dickinson & Co., Rutherford, NJ). The plasma and organs were immediately stored at −70°.

Analytical procedure. The analyses were performed using capillary column gas chromatography–mass spectrometry (GC–MS). A computer controlled LKB 2091 gas chromatograph–mass spectrometer was used.

A detailed description of the analytical procedure used for urine samples has been published previously [7]. Pieces of about 0.2 g of liver, kidney, brain and faeces were homogenized in 1.0 ml of 0.4 M $HClO_4$ containing 28.3 pmoles of 6OMTHBC-$^2H_4$ using an Ultra-Turrax homogenizer. Samples of plasma (1.0 ml) were mixed with 0.5 ml of 0.4 M $HClO_4$ containing 28.3 pmoles of 6OMTHBC-$^2H_4$ using the Ultra-Turrax homogenizer.

The samples were centrifuged at 70,000 g for 15 min. and the supernatants were transferred to new tubes. The samples were prepared for enzymatic hydrolysis (sulphatase containing β-glucuronidase activity) and determination of total 6OMTHBC by adjusting the pH to 5–7 by addition of NaOH. The determination of free and total 6OMTHBC was then performed according to the method previously reported for urine [7].

Quantitation. Calibration curves were constructed

by plotting the peak height ratios (m/z 494/498) of standard samples against the 6OMTHBC concentration. The 6OMTHBC levels were then determined from the peak height ratios of each sample by reference to the calibration curve.

RESULTS

Identification of 6OMTHBC

The identification of 6OMTHBC-$(PFP)_2$ in the extracts was performed by monitoring ion intensities at mass numbers corresponding to the molecular ion (m/z 494) and a characteristic fragment (m/z 479), formed by loss of the C_1-methyl group. The criteria for a positive identification was the presence of a peak at the same retention time and with the same ion intensity ratio (m/z 479/494) as authentic compound. The retention time was determined relative to the internal standard which, under the conditions used, eluted ~1/100 of a minute prior to authentic 6OMTHBC-$(PFP)_2$. This procedure allows a precise determination of the relative retention time.

The chromatograms obtained from the analyses of rat samples (Fig. 1) showed that a compound was present in urine, faeces, plasma, liver and kidney, which had the same characteristics as authentic 6OMTHBC-$(PFP)_2$. In brain, no 6OMTHBC could be detected (<0.3 pmoles/g). It should be noted that the internal standard gives rise to a peak at m/z 479 (M-F) with a relative intensity of 2.5% (m/z 479/483). This disturbs the determination of 6OMTHBC-$(PFP)_2$ at m/z 479, when a high gain is used (Fig. 1b).

Artifactual formation

The possible artifactual formation of 6OMTHBC,

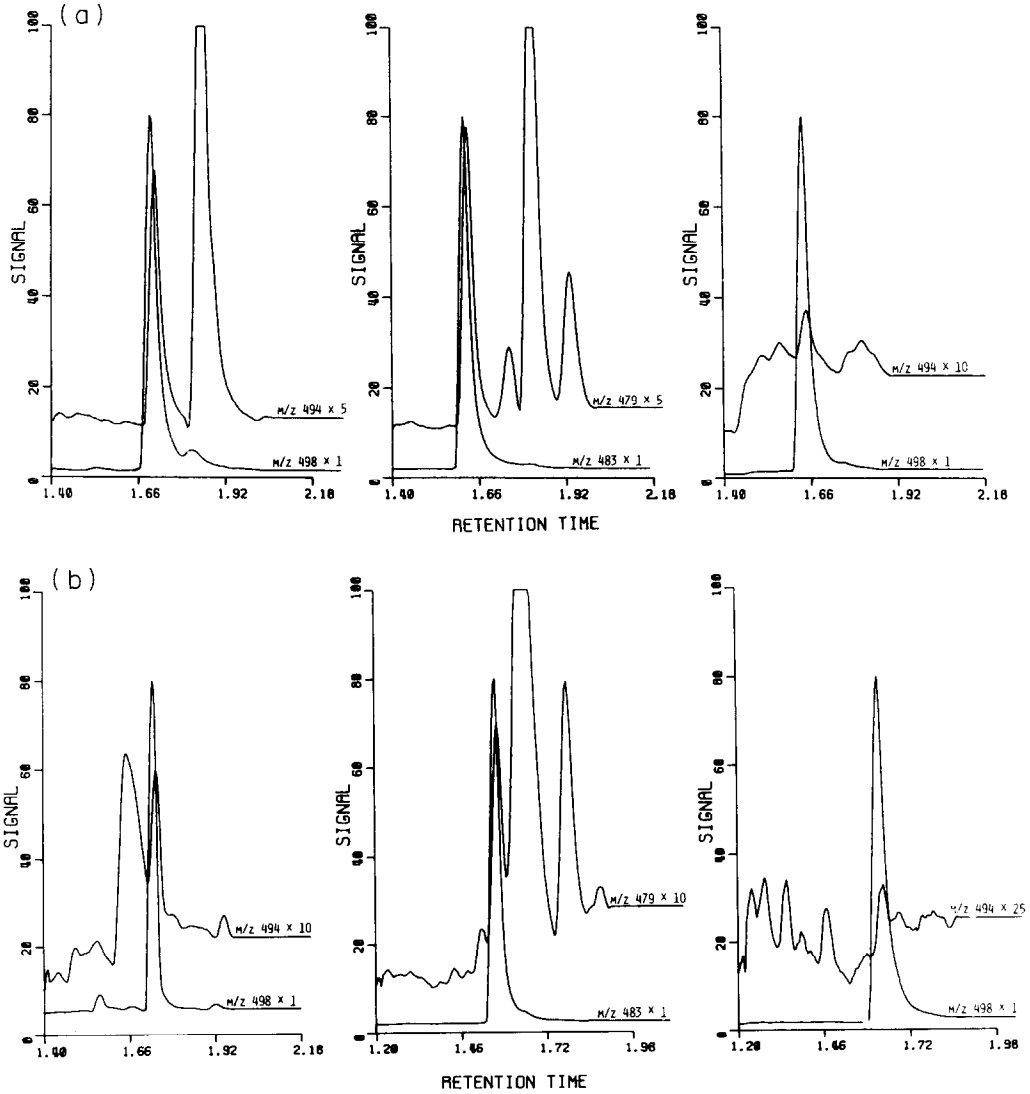

Fig. 1. Chromatograms obtained from the analysis of rat urine (a) and liver (b). For each specimen results from the determination of total 6OMTHBC at m/z 494/498 (left) and at m/z 479/483 (middle), and of free 6OMTHBC at m/z 494/498 (right) are presented. Relative amplification factors are indicated. Retention time is expressed in minutes.

from serotonin and acetaldehyde, was envisaged and studied in two ways. One way was to work up an aqueous solution of 5HT creatinine sulphate (1.0 ml containing 5.4 nmoles), both with and without enzymatic hydrolysis. Another test was to add 5HT-$^2H_4$ instead of 6OMTHBC-$^2H_4$ to rat samples prior to workup and monitor for any formed 6OMTHBC-$^2H_4$. No evidence of artifactual formation was, however, observed. The storage of samples in a frozen state did not affect the 6OMTHBC levels.

Quantitation of 6OMTHBC

The quantitation of 6OMTHBC utilized the ion intensities monitored at m/z 494/498. The calibration curves showed a linear relationship of the peak height ratio m/z 494/498 to the concentration of 6OMTHBC in the range of 0–400 pmoles/sample.

The reproducibility of individual 6OMTHBC analysis was determined in the low concentration range by multiple ($n = 8$) analysis of a pool of rat plasma. The level of 6OMTHBC in the pool was found to be 0.45 ± 0.07 (S.D.) pmoles/ml, which demonstrates a precision of ± 16%. The precision of the method at a higher concentration range was better than ± 5%, as reported previously [7].

The mean excretion rate of total 6OMTHBC in rat urine was 0.73 nmoles/24 hr (Table 1). Most of this was in a conjugated form. The excretion rate in faeces was 25% of that in urine (Table 1). Most of the 6OMTHBC in faeces was in a free form. The mean concentration of 6OMTHBC in the rat urine was 79 ± 24 pmoles/ml

In liver, kidney and plasma, trace amounts (pmoles/g or ml) of 6OMTHBC were present (Table 1). Liver contained the highest amount. In liver, the free compound was measurably only in 3 of 10 samples. In these 3 samples, the free compound amounted to 3.5–8% of the total amount. In kidney, the free compound amounted to 28% of the total, and in plasma to 38%. The concentration ratio of total 6OMTHBC between urine and plasma was about 150.

Elimination and distribution of administered 6OMTHBC

When 6OMTHBC (16.8 nmoles) was injected (i.p.) to rats, 75% of the injected amount was recovered in the urine excreted during the first 10 hr. A larger fraction of 6OMTHBC was found in a free form immediately after injection than later on. In plasma, the concentration of 6OMTHBC declined with a half life of 1.5 hr (Fig. 2a). The concentration ratio of 6OMTHBC as calculated on a per ml basis between urine and plasma was in the order of 100 during the elimination. Nine hr after injection, the plasma concentration of 6OMTHBC was only a few times higher than the endogenous level.

Table 1. Endogenous 6OMTHBC in rat tissues and fluids

	Total	Free	*n*
Liver*	11.1 ± 3.6	<0.5–1.5†	10
Kidney*	2.1 ± 0.9	0.6 ± 0.1	9
Plasma*	0.52 ± 0.15	0.20 ± 0.02	12
Urine‡	0.73 ± 0.20	0.069 ± 0.015	12
Faeces‡	0.18 ± 0.03	0.14 ± 0.03	11

* Expressed as pmoles/g or ml ± S.E.M.
† Detectable in three samples.
‡ Expressed as nmoles excreted/24 hr ± S.E.M.

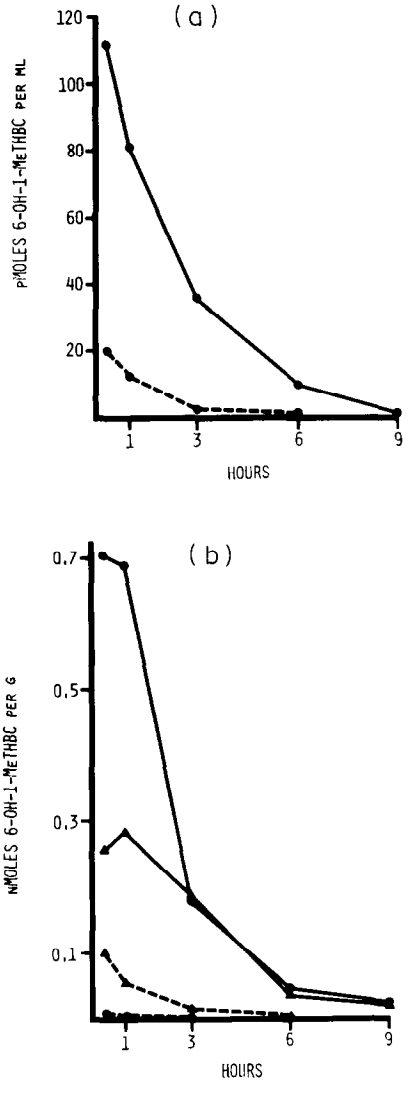

Fig. 2. Levels of total (—) and free (---) 6OMTHBC in (a) rat plasma, and (b) in rat liver (●) and kidney (▲) after i.p. injection of 16.8 nmoles. Each point represents the mean of two determinations.

The level of 6OMTHBC in liver declined with a half life of 1.3 hr and in kidney with a half life of 1.9 hr (Fig. 2b). Only a minute fraction was in a free form in liver. After 9 hr the liver and kidney concentrations had decreased to levels close to the endogenous.

DISCUSSION

This study demonstrates that 6OMTHBC is a normal constitutent or rat tissues and body fluids. This result is in agreement with previous investigations

of 6OMTHBC and the related compound 1MTHBC in man [6, 7]. The concentration of 6OMTHBC in the urine of rat and man [7] is similar, with the difference that a larger fraction is conjugated in the rat. The present study also showed that a significant amount of 6OMTHBC is excreted via faeces. The administration of 6OMTHBC to the rats showed that it is effectively eliminated and metabolically stable. Only trace amounts of 6OMTHBC was present in liver, kidney and plasma. The inability to detect 6OMTHBC in brain tissue, which was reported recently [10], could be confirmed.

The possibility of artifactual formation is of major concern in the field of THBCs. That this is a serious problem for the formaldehyde derived THBCs has been demonstrated [11] in a recent report, which questioned earlier investigations concerning the natural occurrence of formaldehyde derived THBCs. One major pitfall was due to a release or formation of formaldehyde from tissue during homogenization. Artifactual formation seems to be a less serious problem for the acetaldehyde derived THBCs. The work on 6OMTHBC and 1MTHBC in this laboratory [6, 7] has shown that no artifactual formation occurs when pure solvents and reagents are being used. Faull *et al.* [10] have come to the same conclusion.

The significance of 6OMTHBC is unknown. However, the THBCs display a variety of biochemical, pharmacological and behavioural effects [2, 12]. The origin of 6OMTHBC is also unknown. A dietary origin must be considered since 6OMTHBC is present in various dietary components [13]. However, analysis of rat food ruled out this possibility for the rat. It must be concluded, therefore, that 6OMTHBC is formed in the rat body. Although THBCs are easily formed from indoleamines and aldehydes in a Pictet–Spengler reaction [14], other routes might be considered.

Acknowledgements—This work was supported by grants from the Swedish Medical Research Council (04041) and the National Institute of Health (MH 12007).

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