6-METHOXY-1,2,3,4-TETRAHYDRO-β-CARBOLINE IN PINEAL GLAND OF CHICKEN AND COCK

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1. Introduction

In several reports dealing with the pineal gland, mention has been made of an unidentified strongly fluorescent compound occurring in amounts corresponding to the concentrations of melatonin in this gland [1,2]. The potent pharmacological actions of pineal gland extracts on the hormonal function are not easily explained by melatonin or any other of the compounds so far identified from the extract. Interestingly, the fluorescing compound considered responsible for some of these glandular effects has been suggested to possess a β-carboline structure [1,2].

Up to now at least 9 β-carboline derivatives have been reported in the tissues of man or animals [3]. A β-carboline compound in rat arcuate nucleus have been tentatively localized by using laser fluorometry and a 6-methoxy-1,2,3,4-tetrahydro-β-carboline (6-MeO-THBC) structure have been suggested for this compound [4]. At a later stage this compound has been detected in the adrenal gland and brain of rats in amounts of 1.1 μg and 36 ng/g tissue, respectively [5]. This paper describes the presence of 6-MeO-THBC in pineal gland of chicken and cock on the basis of evidence from mass spectrometric studies.

2. Materials and methods

The pineal glands were obtained from 9 male chickens (1.5 months of age) and 10 cocks (8 months of age), White Leghorn. The animals were maintained in a 12-12 lighting regime with normal food and water ad libitum. The chickens were decapitated between 12.00–16.00 h. The cocks were decapitated in 2 groups: 5 in the morning at the beginning of the light period (at 7.00 h) and 5 in the evening at 18.00 h. After weighing the glands were frozen to −80°C. The investigation was carried out in 2 parts: The pineal glands of chickens were examined in 3 groups each containing 3 glands while the glands of cocks were examined separately. The samples were homogenized in 0.1 ml of 0.1 M HCl containing the isotopically pure deuterated analog of 6-MeO-THBC as internal standard and semicarbazide for the binding of free aldehydes. After centrifugation the supernatants were separated, and the sediments were washed twice with 0.2 ml of semicarbazide containing distilled, ion-free water. The water phases were made alkaline with a saturated solution of Na_2CO_3 (after addition of NaCl) and extracted 3 times with 2.5 ml of cold freshly distilled aldehyde-free diethylether. After evaporation the residues were treated with heptafluorobutyryl (HFB) imidazole and analyzed by the gas chromatographic-mass spectrometric method as in [6]. To verify that 6-MeO-THBC was not formed during the analytical procedure, isotopically pure [α,α,β,β-$^2H_4$]-5-methoxytryptamine (d_4-5-MeO-TA) was added to some of the samples.

The 6-MeO-THBC, the internal standard 3,3,4,4-[$^2H_4$]-6-methoxy-1,2-dihydro-β-carboline (d_4-6-MeO-THBC) and d_4-5-MeO-TA were synthetized in this laboratory. The isotopic purity, determined by mass spectrometry, was greater than 98%. The heptafluorobutyryl imidazole was obtained from Pierce Chemical Company (Rockford, IL USA). All reagents were of analytical grade, and all solvents were redistilled before use. The free aldehydes were removed from the solvents by shaking with semicarbazide [7].

3. Results

The identification of 6-MeO-THBC in samples was based on the mass spectra (fig.1) or on monitoring the ions *m/e* 398, 229, 201, 185 and 173 (fig.2) and on the GLC retention, which was identical with that of the internal standard. The molecular ion M^+ is the base peak (*m/e* 398 for 6-MeO-THBC and *m/e* 402 for internal standard). Other intense peaks in the spectrum of 6-MeO-THBC occur at *m/e* 229, 201,

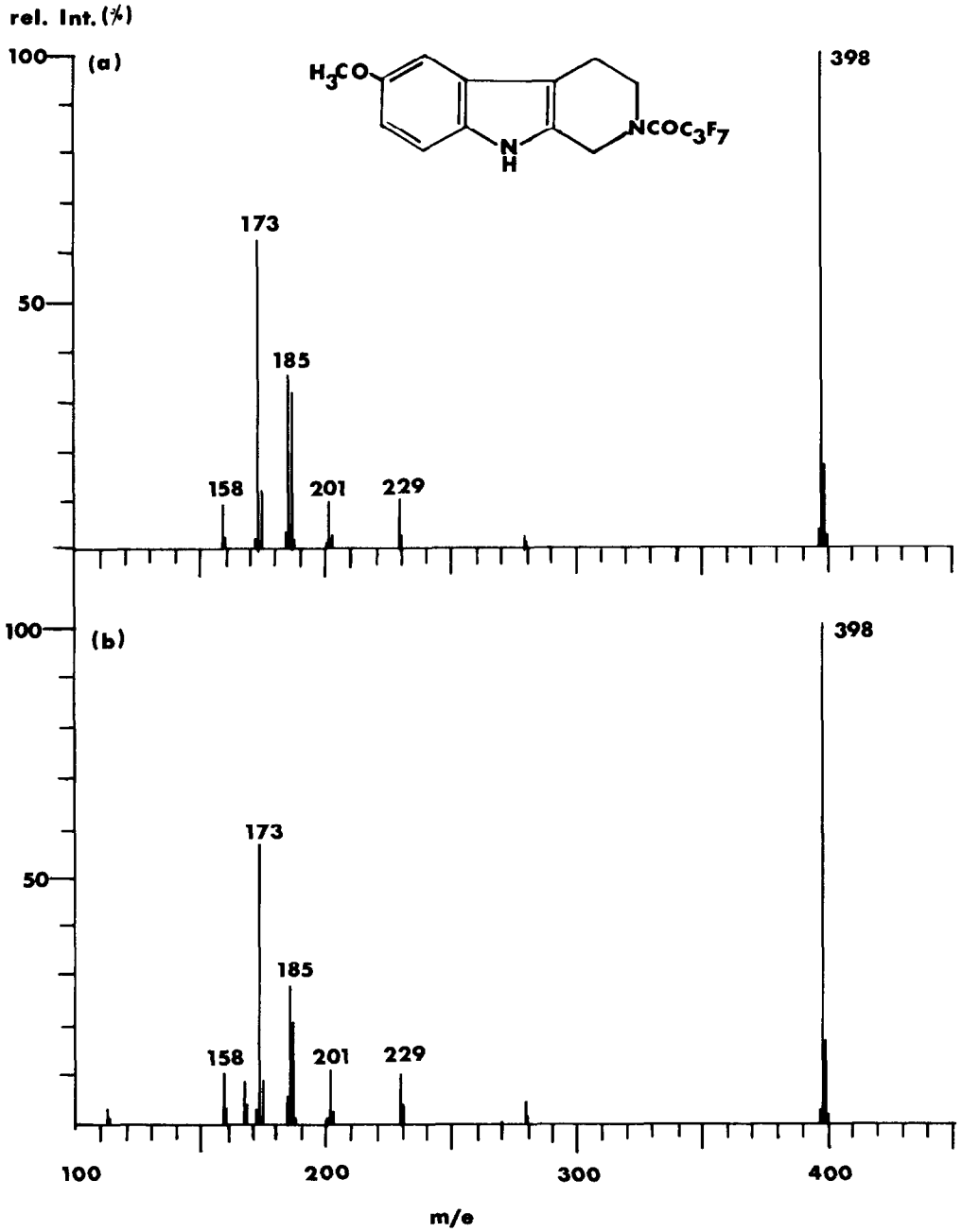

Fig.1. Mass spectra of heptafluorobutyryl derivatives of (a) authentic 6-methoxytetrahydro-β-carboline (6-MeO-THBC) and (b) 6-MeO-THBC extracted from pineal gland of chicken (ionization energy 23 eV).

Table 1
The concentrations of 6-methoxytetrahydro-β-carboline (6-MeO-THBC) in chicken pineal glands

Sample	Time of decapitation	6-MeO-THBC (nmol/g)	Weight of glands (mg)
1	12.00	29.4	12.0
2	14.00	43.0	12.3
3	16.00	8.5	12.8

Each sample consists of three glands

386, 185, 173 and 158. These specific ions show identical relative intensities in the spectra of authentic and extracted 6-MeO-THBC.

The peak height ratio of the molecular ions of 6-MeO-THBC and internal standard (*m/e* 398/402) was used in the quantitation. The selected ion monitoring indicated that high concentrations of 6-MeO-THBC are present in all samples examined (tables 1,2). 6-MeO-THBC concentrations are higher in the pineal glands of chickens than in those of cocks, and the concentrations seems to decrease during the light period.

The addition of d_4-5-MeO-TA into some samples during the homogenization step did not lead to detectable formation of d_4-6-MeO-THBC. Consequently, 6-MeO-THBC is an endogenous compound in chicken and cock.

4. Discussion

The results of the present investigation show that 6-MeO-THBC is a normal constituent in the pineal gland of chicken and cock. The site of biosynthesis of this compound remains to be studied. However, the high 6-MeO-THBC concentrations in the pineal gland indicate that the synthesis of this compound may occur in this gland. The levels of 6-MeO-THBC are of the same order as those of melatonin in the pineal gland [8], however, some of its pharmacological actions are more prominent than those of melatonin.

Circadian rhythms have been demonstrated in the biochemical processes of pineal gland of birds and mammals. 3–5-fold changes occur in avian pineal serotonin [9] and melatonin levels [10]. The changes in melatonin levels may have a direct relationship to pineal *N*-acetyltransferase activity [11]. Although here the 6-MeO-THBC levels in the cocks are measured at 2 points of the light period only, the results seem to indicate a comparable light-dark cycle. Hydroxy-*O*-methyltransferase may have a role in the regulation of this cycle.

Much evidence has been accumulated to indicate that tetrahydro-β-carbolines (THBCs) are naturally

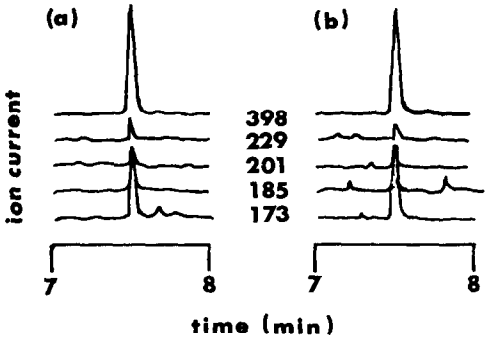

Fig.2. Selected ion recordings of the ions at *m/e* 398, 229, 201, 185 and 173 for heptafluorobutyryl derivatives of (a) pure 6-methoxytetrahydro-β-carboline (6-MeO-THBC) and (b) 6-MeO-THBC extracted from pineal gland of chicken.

Table 2
The concentrations of 6-methoxytetrahydro-β-carboline (6-MeO-THBC) in cock pineal glands

Gland	6-MeO-THBC (nmol/g)	Weight of glands (mg)
1	1.4	3.7
2	6.4	3.2
3	0.8 [a] 3.3 ± 1.2	4.3
4	6.0	3.1
5	2.0	3.7
6	1.1	3.2
7	0.8	3.7
8	1.3 [b] 1.1 ± 0.2	3.5
9	0.4	3.4
10	1.8	3.8

Glands 1–5 were collected at 7.00 h in the morning at the beginning of light period and the glands 6–10 at 18.00 h. [a] and [b], Mean ± S.E.

occurring compounds in human and animal tissues [3,4,6,12]. The possible role of THBCs as neuromodulators has been discussed in [3,5,6,12]. THBCs have been shown to have direct influence on neurotransmitters, especially on 5-hydroxytryptamine, by acting on receptors [13], uptake [14], release [15] and catabolism [16] of these compounds. On the other hand, the endocrinological effects of THBCs are less known. However, 6-MeO-THBC has been reported to cause an initial increase in the diestrus followed by a total disappearance of the estrus cycle in rats [13]. A significant increase in serum prolactin level was obtained after a single dose of 6-MeO-THBC [17]. Thus, in addition to its effects on neurotransmitters, 6-MeO-THBC of the pineal gland also seems to have a hormonal function in the release of the other hormones under pineal control.

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