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6-Methoxy-tetrahydro-β-carboline and Melatonin in the Human Retina

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The presence of two 5-methoxyindoles, 6-methoxy-1,2,3,4-tetrahydro-β-carboline (6-MeO-THBC) and melatonin was demonstrated in human retinae using a highly specific gas chromatographic mass spectrometric method from eyes affected with various ocular diseases. Both compounds were found to occur in similar quantities. 6-MeO-THBC is a newly-identified endogenously-occurring retinal compound possibly acting as a neuromodulator. The importance of 6-MeO-THBC and other β-carbolines especially compared to other 5-methoxyindoles is discussed.

Key words: β-carbolines; melatonin; methoxyindoles; gas chromatography; mass spectrometry; retina; man.

1. Introduction

Of all the 5-methoxyindoles, melatonin has been most extensively studied. Melatonin has been shown to exist in various extrapineal sources, in the retina (Pang, Brown, Grota, Chambers and Rodman, 1977; Hamm and Menaker, 1980; Pang, Yu, Suen and Brown, 1980; Yu, Pang and Tang, 1981), the Harderian gland, brain and serum of rats and chicken (Pang et al., 1977). The enzymes N-acetyltransferase (NAT) and hydroxyindole-O-methyltransferase (HIOMT) responsible for the formation of melatonin have been identified in the retina (Quay, 1965; Nagle, Cardinali and Rosner, 1972; Hamm and Menaker, 1980; Pevet, Balemans, Legerstee and Vivien-Roels, 1980). Retinal synthesis of melatonin has been suggested (Gern and Ralph, 1979). 6-MeO-THBC (5-methoxytryptoline) has been identified in the rat brain and adrenal gland (Barker, Harrison, Monti, Brown and Christian, 1981), the pineal gland of fowl and humans (Kari, Airaksinen, Gynther and Huhtikangas, 1983) and in the retina of some mammals (Leino, Kari, Airaksinen and Gynther, 1983).

2. Materials and Methods

Retinas of 11 human eyes were obtained at enucleations performed in cases of severe eye disease. Presumably healthy pieces of retina were obtained from two eyes in cases of intraocular melanomas and in two cases of intraorbital tumors. Other retinas suffering a varying degree of destruction were from eyes afflicted with sequelae of severe perforating injuries, painful absolute glaucomas with deformation of bulbi, a diabetic complication and one eye from a severely debilitated person with a pseudomonas perforated infection. Variable sized pieces of retina were detached immediately after the enucleation and the retinas were kept in deep freeze (−70°C) until further procedures.

The samples were processed as described earlier (Leino et al., 1983). In brief, the samples were homogenized in 0·1 M HCl containing isotopically pure 6-methoxy [3,3,4,4-d_4]1,2,3,4-tetrahydro-β-carboline (d_4-6-MeO-THBC) and N-acetyl[α,α,β,β-d_4]5-methoxytryptamine (d_4-melatonin) as the internal standards. The samples were centrifuged, washed, alkalized,

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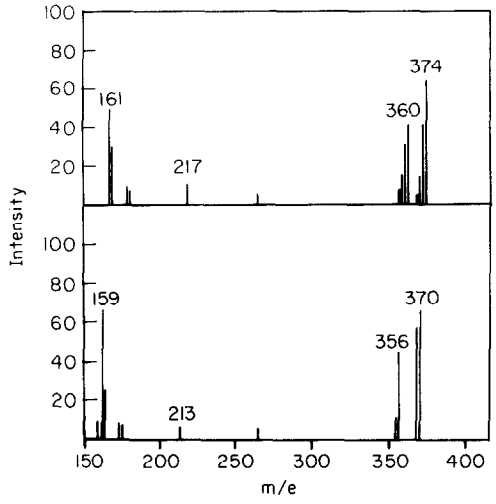

FIG. 1. The mass spectra of the heptafluorobutyrylimidazole derivatives of d_4-melatonin (upper trace) and authentic melatonin (lower trace). The mass to charge ratio (m/e) is plotted against signal intensity.

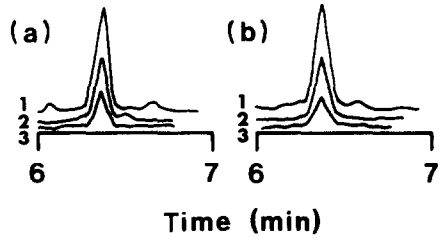

FIG. 2. Selected ion recordings of heptafluorobutyrylimidazole derivatives of (a) d_4-melatonin and (b) melatonin extracted from retinae. m/e a: (1) 374, (2) 360 and (3) 217 and m/e b: (1) 370, (2) 356 and (3) 213.

extracted and the extracts were derivatized with heptafluorobutyrylimidazole and the 6-MeO-THBC residues were analyzed by the gas chromatographic mass spectrometric method described previously (Kari, Peura and Airaksinen, 1980; Kari, 1981; Leino et al., 1983). The identification of 6-MeO-THBC in the samples was based on monitoring the ions at m/e 398, 185, and 173 and observing the retention times in gas liquid chromatography which were identical to those of the internal standard. Similarly for melatonin we observed the ions at m/e 370, 356 and 213 for which the retention times were identical with the ions of the internal standard at m/e 374, 360 and 217 respectively. The ratio of the ion m/e 398 and the corresponding ion m/e 402 of the internal standard were used for quantitation of 6-MeO-THBC. The ratio of the ion m/e 370 and the corresponding ion m/e 374 of the internal standard were similarly used for quantitation of melatonin. The 6-MeO-THBC and the internal standards (d_4-6-MeO-THBC) and (d_4-melatonin) were synthesized in our laboratory.

For comparison we also determined the quantity of 6-MeO-THBC in two samples of platelet-rich plasma using the same methodology described above.

3. Results

The mass spectrum of 6-MeO-THBC was described in our earlier report (Leino et al., 1983). The mass spectra of d_4-melatonin and authentic melatonin are shown in Fig. 1. The retention times of the various ions of 6-MeO-THBC and melatonin extracted from retinal tissue and their respective deuterated analogues were identical

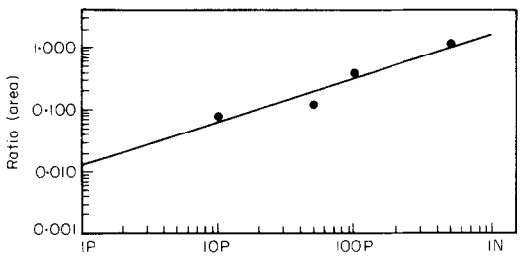

FIG. 3. Calibration curve for quantitative determination of melatonin (m/e 370/374). The numbers of picograms are plotted against the normalized areas of the recorded peaks.

TABLE I

The amount of 6-MeO-THBC and melatonin in each retinal sample in pg mg^{-1} of wet weight of tissue. (Age of patient in parenthesis)

Sample	Diagnosis	6-MeO-THBC	Melatonin
1	Orbital carcinoma (60)†	3·72	*
2	Intraocular melanoma (62)†	1·53	1·46
3	Intraocular melanoma (58)†	1·06	4·64
4	Orbital lymphatic tumour (74)†	0·98	5·71
5	Inflamed eye after perforation (45)	3·78	10·10
6	Infected eye after pseudomonas perforation (50)	0·28	0·75
7	Hemorrhagic absolute glaucoma (83)	1·96	2·20
8	Traumatic secondary absolute glaucoma (11)	0·78	5·75
9	Diabetic phthisic eye (47)	1·75	*
10	Perforated infected eye with foreign bodies (27)	*	3·70
11	Painful absolute glaucoma (80)	*	5·62
	Mean	1·76	4·43
	S.E.M.	0·41	0·94

* Not measured.
† Sample consists of macroscopically and obviously also functionally healthy retina.

(Fig. 2). The mass spectrometer was calibrated daily and a typical calibration curve for melatonin is shown in Fig. 3.

The amounts of 6-MeO-THBC and melatonin in the retinal samples were of a similar quantitative order (Table I). It is noteworthy that these compounds were found also in eyes with non-healthy retinal elements. These injured or diseased eyes invariably had a very disorganized intrabulbar appearance and the samples always contained substantial amounts of tissues such as blood, cellular debris, fibrotic tissue, chorioidea and clouded cellular vitreous.

The amount of 6-MeO-THBC in platelet-rich plasma was less than 0·5 pg mg^{-1} of blood in both samples and thus the blood-borne 6-MeO-THBC cannot possibly account for the retinally-occurring 6-MeO-THBC.

4. Discussion

In an earlier study we showed the presence of 6-MeO-THBC in the retinas of pigs and rabbits with considerably lower concentrations in pig retinas (Leino et al., 1983). Our present results show that human retinal 6-MeO-THBC appears in quantities of the same order as in the pig retina. The presence of these compounds also in eyes having

suffered obvious retinal destruction suggests that possibly the retinal vascular and other disease-induced elements contributed a share of these results. Some investigators have observed that after perfusion of retina to remove blood the amount of rabbit retinal 5-hydroxytryptamine reduced to about one-sixth from the non-perfused retinal concentration (Ehinger, Hansson and Törnqvist, 1981). Comparing the amount of 6-MeO-THBC in blood and in the retinal samples we assume that the blood does not account for much of the retinal 6-MeO-THBC. The concentration of retinal 6-MeO-THBC and melatonin fall far short of pineal concentrations. In this experiment the intense operating-room lighting might have an effect on the results.

Melatonin has been shown to occur in retinas of various animals and recently even a retinal synthesis of melatonin has been proposed (Gern and Ralph, 1979). The origin of retinal 6-MeO-THBC seems to be mostly pineal but our preliminary investigations using deuterated 5-methoxytryptamine as a precursor and observing the respective deuterated 6-MeO-THBC in the retina suggest that a fraction of the retinal 6-MeO-THBC is locally synthesized (unpubl.).

An active retinal uptake mechanism for 6-MeO-THBC has been suggested earlier (Thomas, Buckholtz and Zemp, 1979) and our results with 6-MeO-THBC autoradiography (unpubl.) support this hypothesis.

Of the 5-methoxyindoles, melatonin has been studied by far the most, nevertheless not much of its retinal function is known. It has been suggested that melatonin regulates the amount of light reaching the photoreceptor cells (Pang and Yew, 1979). Immunohistochemical studies have shown that melatonin is localized in the outer nuclear layer of the retina in rat (Bubenik, Brown and Grota, 1976) and fish, reptiles and hamsters (Vivien-Roels, Pevet, Dubois, Arendt and Brown, 1981). The retinal melatonin occurrence has been shown to be strongly scotophasic by radioimmunoassay and immunohistological methods (Bubenik, Purtill, Brown and Grota, 1978; Hamm & Menaker, 1980; Pang et al., 1980; Yu et al., 1981). A similar strongly scotophasic circadian rhythm was shown for NAT with a 15-fold concentration at dark compared to the daytime (Hamm and Menaker, 1980) and thus NAT may be a rate limiting enzyme in the circadian synthesis of melatonin. Some have shown a circadian rhythm in retinal HIOMT (Nagle et al., 1972) while others have observed no substantial fluctuation (Wainwright, 1979; Pevet et al., 1980); furthermore Nagle, Cardinali and Rosner (1973) observed that pinealectomy abolished the circadian rhythm of retinal HIOMT. A strong scotophasic fluctuation of melatonin (Yu et al., 1981) and NAT (Hamm and Menaker, 1980) has been shown to persist even after pinealectomy. The day–night fluctuation of pineal and blood melatonin has been well documented with high concentrations always at night in both nocturnal and diurnal animals (for references see Reppert and Klein, 1980).

The possible physiological role of 6-MeO-THBC has so far received very little attention and in particular its role in retinal physiology is comletely unknown. Some of the established effects of 6-MeO-THBC are the inhibition of 5-HT uptake and monoamine oxidase A activity and a melatonin-like antigonadotrophic effect (see Airaksinen and Kari, 1981). Both the pineal and the extrapineal 5-methoxyindoles seem to have an effect on the gonadal axis possibly via the environment e.g. the light conditions.

Presently the function of human melatonin or other 5-methoxyindoles including the β-carbolines is not known but for the time being they can be regarded as potential hormones. The β-carbolines have also been proposed as neuromodulators (Barchas, Akil, Elliott, Holman and Watson, 1978). It is interesting to notice the nearly similar

concentrations of both studied 5-methoxyindoles and this fact considerably raises the investigative zeal towards the retinal β-carbolines.

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