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ANALYSIS OF MELATONIN, 5-METHOXYTRYPTOPHOL AND 5-METHOXYINDOLEACETIC ACID IN THE PINEAL GLAND AND RETINA OF HAMSTER BY CAPILLARY COLUMN GAS CHROMATOGRAPHY—MASS SPECTROMETRY

OLOF BECK*,*

Department of Toxicology, Karolinska Institutet, Box 60 400, S-104 01 Stockholm (Sweden)

and

PAUL PÉVET

Zoological Laboratory, University L. Pasteur, Strasbourg (France) and The Netherlands Institute for Brain Research, Amsterdam (The Netherlands)

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SUMMARY

A specific capillary column gas chromatographic—mass spectrometric method was used to determine 5-methoxyindoles in the pineal gland and retina of the golden hamster during a light—dark (14:10) cycle. In the pineal gland, the mean levels of melatonin ranged from 0.15 to 2.4 pmol per gland, with a maximum in the dark. The levels of 5-methoxytryptophol and 5-methoxyindoleacetic acid were in the same range, but peaked during light. In the retina the levels of melatonin were about 100 pmol/g, and seemed not to differ between light and dark. The level of 5-methoxyindoleacetic acid were in the same range during light but were below the detection limit during dark.

INTRODUCTION

Interest in 5-methoxyindoles has so far mainly been focussed on melatonin and its function as a pineal hormone [1]. Although another 5-methoxyindole,

*Correspondence address: Department of Psychiatry and Behavioral Sciences, Stanford University Medical Center, Stanford, CA 94305, U.S.A.

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5-methoxytryptophol (5MTOL), has long been known to exert an influence on reproduction [2], it is only recently that the existence and physiological effects of 5-methoxyindoles other than melatonin have gained interest [3, 4]. In addition, several findings have demonstrated that organs other than the pineal gland (e.g. the retina) possess the capability to produce 5-methoxyindoles (see ref. 5).

The aim of the present study was to determine endogenous levels of melatonin, 5MTOL and 5-methoxyindoleacetic acid (5MIAA) in the pineal gland and retina of the golden hamster during a light—dark cycle, by using a specific capillary column gas chromatographic—mass spectrometric (GC—MS) method.

EXPERIMENTAL

Chemicals and biological samples

5MTOL was obtained from Sigma (St. Louis, MO, U.S.A.); melatonin was from Regis (Morton Grove, IL, U.S.A.); 5MIAA was from Aldrich (Beerse, Belgium); pentafluoropropionic anhydride (PFPA) was from Reagenta (Uppsala, Sweden); and 2,2,2-trifluoroethanol (TFE) was from E. Merck (Darmstadt, F.R.G.). 5-Methoxyindole-3-[2-$^2H_2$]acetic acid (5-[$^2H_2$]MIAA) was synthesized according to the procedure of Beck and Bosin [6]. 5-Methoxy-[$\alpha,\alpha,\beta,\beta$-$^2H_4$]tryptophol (5-[$^2H_4$]MTOL) was synthesized by the method of Hesselgren and Beck [7]. N-Acetyl-5-methoxy-[$\alpha,\alpha,\beta,\beta$-$^2H_4$]tryptamine ([$^2H_4$]-melatonin) was prepared by the procedure of Shaw et al. [8]. All other chemicals used were of analytical purity.

Male golden hamsters (*Mesocricetus auratus*) (80—90 g) were obtained from TNO (Zeist, The Netherlands). The animals were maintained under a long photoperiod (light—dark 14:10), with the light on between 4.00 a.m. and 6.00 p.m., at 25°C in constant humidity; they received food and water ad libitum. After decapitation, the pineal gland and retinae were quickly removed. The tissues were frozen in liquid nitrogen and stored at —70°C prior to analysis. During the night the animals were killed in the dark, but dissected in the light.

Preparation of samples

The pineal glands from three animals were pooled and the retinae from each animal were analysed together. The tissue was homogenized in 1.0 ml of ice-cooled 0.15 *M* formic acid containing [$^2H_4$]melatonin (38.7 pmol), 5-[$^2H_4$]-MTOL (74.8 pmol) and 5-[$^2H_2$]MIAA (47.2 pmol), using an Ultra-Turrax homogenizer. The homogenate was centrifuged at 70,000 *g* for 15 min and the supernatant transferred to a clean acid-washed (dichromate—sulphuric acid) 15-ml glass-stoppered tube containing 6 ml of dichloromethane. The tube was shaken and centrifuged at 1000 *g* for 5 min. The organic layer was divided into two equal parts which were transferred to new tubes and evaporated to dryness under a stream of nitrogen. One part of the extract was used for the analysis of melatonin and 5MTOL. The extract was treated with 50 μl of PFPA at 60°C for 1 h, evaporated to dryness under nitrogen and redissolved in 25 μl of ethyl acetate. The other part of the extract was used for the analysis of

5MIAA. This part was treated with 50 μl of a mixture of PFPA and TFE (4:1) at 75° C for 5 min, followed by evaporation to dryness under nitrogen. Thereafter, the residue was treated as described above for the melatonin and 5MTOL fraction.

Gas chromatography—mass spectrometry

Selected ion monitoring was performed using a computer-controlled LKB 2091 gas chromatograph—mass spectrometer. The gas chromatograph and the mass spectrometer were interfaced with a jet separator. The GC separations were achieved using a 25 m × 0.32 mm I.D. WCOT SE-52 capillary column and helium was used as carrier and make-up gas. Splitless injections were carried out using a "moving needle" device. The GC conditions were: injector heater 260°C; column temperature 200°C for 5MTOL and 5MIAA and 230°C for melatonin; column flow-rate ~2 ml/min and make-up gas flow-rate ~12 ml/min. Aliquots of 2 μl of the samples were injected and an initial delay of about 1.5 min in opening the valve was effected to avoid contamination of the ion source. Under these conditions the retention times of the derivatives of the 5-methoxyindole compounds were about 2 min. The MS conditions were: separator temperature 250°C; ion source temperature 240°C; electron energy 70 eV; and trap current 50 μA. The mass numbers monitored for melatonin, 5MTOL and 5MIAA were *m/z* 360:364, 483:487 or 319:322, and 433:435, respectively, where the lower mass number corresponds to the authentic compound and the higher to the deuterated internal standard.

Quantitation

Calibration curves were constructed by plotting the peak height ratios (authentic/internal standard) of the standard samples against the concentration of authentic compound. The levels were then determined from the corresponding peak height ratios of each sample by reference to the calibration curve. The calibration curves showed a linear relationship of the peak height raios to the concentration and always intercepted near the origin.

RESULTS

Identification

The selected-ion monitoring involved recording ion intensities at mass numbers corresponding to the molecular ions and, in the case of 5MTOL, also to a characteristic fragment ion [9—11]. The identification was based on the presence of compounds eluting at the same retention time as authentic compounds and at the correct mass numbers. Evidence for the presence of melatonin, 5MTOL and 5MIAA in the pineal gland (Fig. 1), and of melatonin and 5MIAA in the retina (Fig. 2), was obtained. The reproducibility of the method was better than 10% for all three compounds.

Pineal levels

During the light phase the level of melatonin in the pineal gland was found to be about 0.15 pmol per gland (Fig. 3). The melatonin level was increased about fifteen-fold to 2.4 pmol per gland during the dark. The levels of 5MTOL

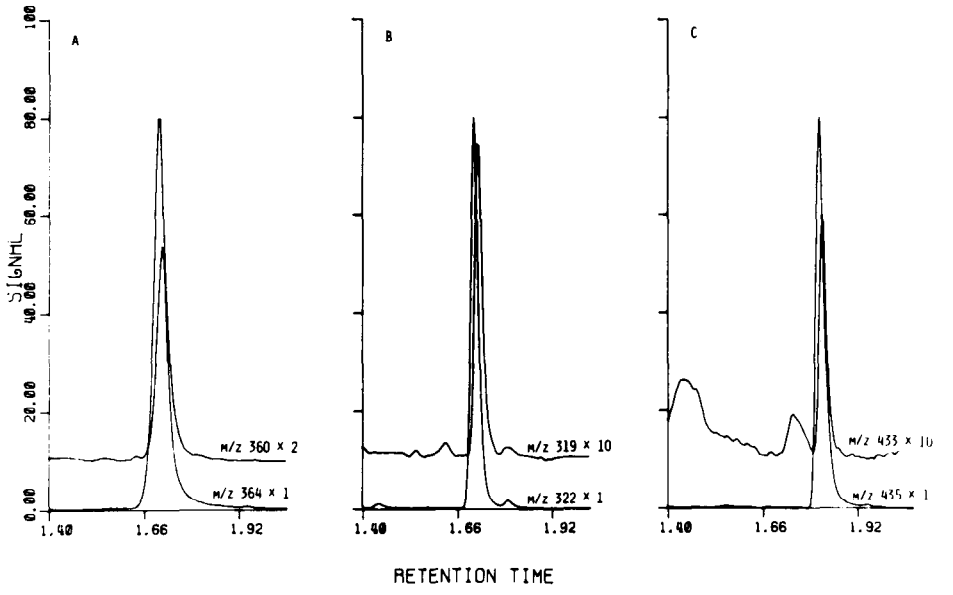

Fig. 1. Ion trace chromatograms obtained from the analysis of (A) melatonin, (B) 5MTOL, and (C) 5MIAA in hamster pineal glands. Mass numbers (m/z) and relative amplification factors are indicated. The retention time is expressed in minutes.

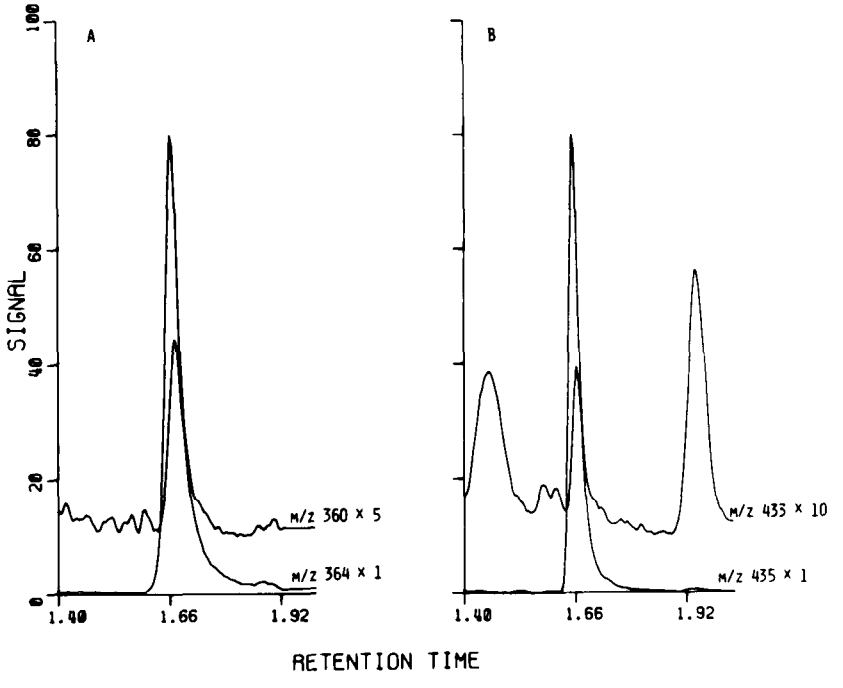

Fig. 2. Ion trace chromatogram obtained from the analysis of (A) melatonin, and (B) 5MIAA in hamster retinae. Mass numbers (m/z) and relative amplification factors are indicated. The retention time is expressed in minutes.

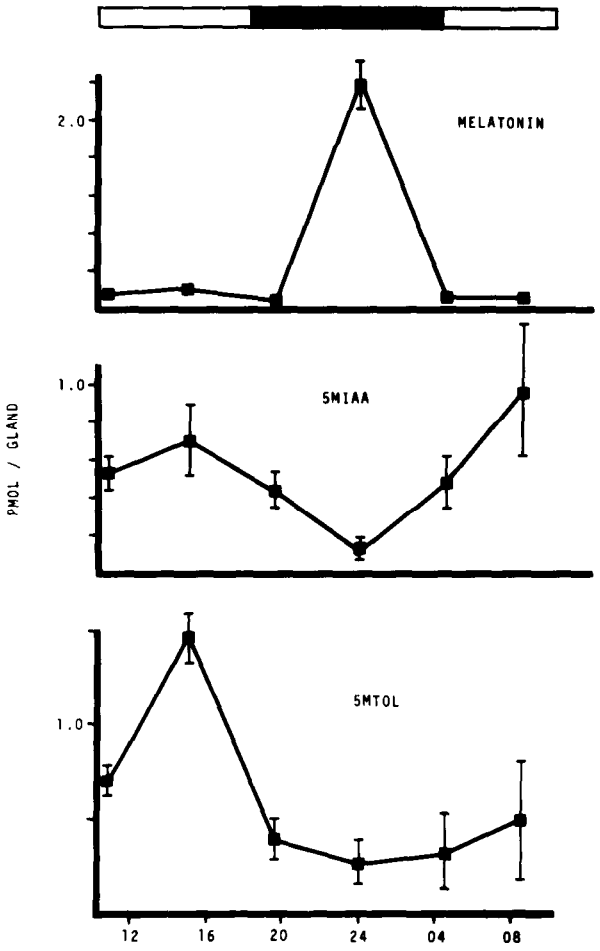

Fig. 3. Concentrations of melatonin, 5MTOL and 5MIAA in the hamster pineal gland during a light—dark cycle (long photoperiod, light—dark 14:10).

and 5MIAA were of a similar magnitude to those of melatonin (Fig. 3). The maximal levels of 5MTOL and 5MIAA occurred, however, during the light phase, and there was less difference than for melatonin between light and dark.

Retina levels

Melatonin was present in the retina at a level of about 100 pmol/g (Fig. 4). No difference in the melatonin level between light and dark phase was evident. 5MIAA was detectable in most samples collected during light and the levels seemed to be at the detection limit during dark (Fig. 4). There was a significant variation in results within the groups. 5MTOL could be detected in a few samples (~10%), with a limit of detection of about 5 pmol/g. There appeared to be a relation between melatonin, 5MIAA and 5MTOL in the retina in that when the melatonin level was high, 5MIAA and 5MTOL also occurred at relatively high levels.

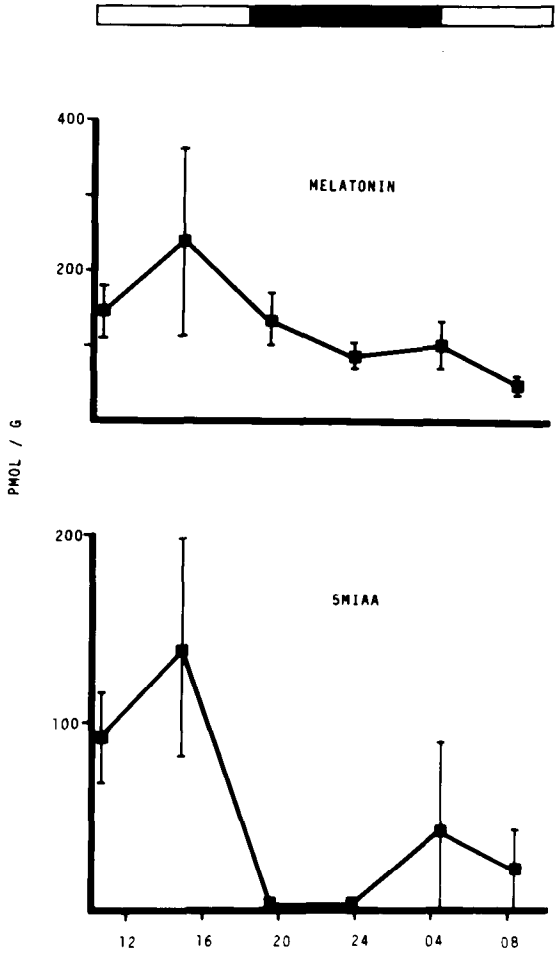

Fig. 4. Concentrations of melatonin and 5MIAA in the hamster retina during a light—dark cycle (long photoperiod, light—dark 14:10).

DISCUSSION

The results of this study, obtained by use of GC—MS, confirm the presence of melatonin in the pineal gland of the male golden hamster, as had previously been found using radioimmunoassay methods [12—14]. The diurnal variation of the melatonin levels in the pineal gland, with a maximum during the dark phase of the light—dark cycle, is also in agreement with the earlier studies. However, some disagreement exists with regard to absolute levels. This study is in accordance with the results of Tamarkin et al. [12] and points to slightly lower levels than reported by others [13, 14].

The presence of melatonin in the retina of both non-mammalian and mammalian species (see ref. 5), including hamster [15], has previously been reported. Our results are in good agreement with those obtained previously in hamster [15]. The absence of a diurnal variation of the melatonin concentration is in agreement with a study of the ground squirrel [16], but in variance

with studies of quail, pigeon, chicken and rat [17—19]. However, our results are in agreement with a study of Pévet et al. [20] in which the capacity of the hamster retina to synthesize melatonin was similar throughout the light—dark cycle.

The presence of 5MTOL and 5MIAA in the pineal gland of hamster is in agreement with earlier studies of other species (see ref. 4). The diurnal variation of the 5MTOL levels, with a maximum during the light phase, is in agreement with an earlier study of the capacity of hamster pineal to synthesize 5MTOL [20]. In rat pineal, however, a maximum in 5MTOL levels during dark has been reported [10, 21].

This study reports for the first time the presence of 5MIAA in retina. The level of 5MIAA appeared to possess a diurnal variation, with high levels during the light phase. However, as for melatonin, there was a significant variation in the results, which may indicate that factors other than light—dark influence its level.

The exact physiological function of the 5-methoxyindoles has yet to be demonstrated. However, both melatonin and 5MTOL possess physiological effects [1, 4]. It is interesting to note that in the pineal gland melatonin and 5MTOL occur at similar concentrations but with maximum levels at different times of the light—dark cycle. In retina, however, melatonin occurs at a substantially higher concentration than 5MTOL. 5MIAA may arise as a metabolite of all the other 5-methoxyindoles [22—24] and as yet no physiological effect has been described for this compound.

In conclusion, the present study has demonstrated that 5-methoxyindoles other than melatonin are present in the pineal gland and retina of hamster, and that their levels possess a diurnal variation. This strengthens the concept that melatonin is not the only 5-methoxyindole that has to be considered when pineal function is studied.

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