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Retinal 5-Methoxytryptamine and 5-Methoxyindole-3-acetic Acid in the Rat and Quail: Diurnal Rhythms and Interspecies Differences

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Endogenous 5-methoxytryptamine (5MT) and its biosynthetic oxidation product, 5-methoxyindole-3-acetic acid (5MIAA), were successfully identified and measured in the retina of the rat and quail by gas chromatography/electron-capture negative ion chemical ionization mass spectrometry (GC/EC-NICI-MS). In the rat retina, diurnal rhythms of 5MT and 5MIAA, with high levels at mid-light and opposite to that of melatonin, were observed. In the quail, high levels of retinal 5MT and 5MIAA were found at mid-dark, and in phase to that of melatonin. Biosynthetic pathways for retinal 5MT and 5MIAA in the rat and quail were discussed in relation to the diurnal rhythms observed. Our results indicate that the biosynthesis and physiological functions of retinal 5MT and 5MIAA could be species dependent.

5-Methoxytryptamine (5MT) and 5-methoxyindole-3-acetic acid (5MIAA) are active biomodulators (1-3). They have inhibitory effects on the reproductive function of a number of animals studied (3,4). In fact, 5MT is a specific agonist for 5HT4 serotonin receptor subtype and may regulate serotonin synthesis, storage and release in many tissues and systems including the retina. It has been shown that 5MT suppressed light-evoked dopamine release and aggregated melanophore pigment granules in the retina of the frog *Xenopus laevis* (5). In cultured neural cells prepared from embryonic chick retina, 5MT inhibited forskolin-stimulated cAMP production via a pertussis toxin-sensitive site (6). Conversely, 5MT increased [^{3}H]-dopamine release from the carp (*Cyprinus carpio*) retina (7), and intracellular cAMP levels in the rabbit retina (8). Unlike 5MT, there is no report of 5MIAA on the retinal function.

5MT and 5MIAA are derivatives of serotonin and metabolites of melatonin (3,9). In the pineal gland, a major biosynthetic pathway of 5MT is direct O-methylation of serotonin by the enzyme hydroxyindole-O-methyltransferase (HIOMT, EC 2.1.1.4), which is in competition with N-acetylation by the enzyme N-acetyltransferase (NAT, EC 2.3.1.5) (3). Pineal 5MT formed is oxidized by monoamine oxidase (EC 1.4.3.4) to yield 5-methoxyindole acetaldehyde, which was further metabolized to 5MIAA by aldehyde dehydrogenase (EC 1.2.1.3) (1,3). Both NAT and HIOMT are present in the retina of vertebrates (10-12). In vitro studies by radio-enzymatic techniques showed that the retina synthesizes 5MT and 5MIAA in similar quantities to those produced by the pineal gland (13). However, the biosynthetic pathways of methoxyindoles in the retina appear to be different from that of the pineal. Within the eye of *Xenopus laevis* and the lizard *Anolis carolinensis* (9), retinal 5MT was shown to be formed from melatonin by the deacetylation action of aryl acylamidase (EC 3.5.1.13). While melatonin deacetylation was further demonstrated in the pineal gland and brain of *Anolis carolinensis* and *Sceloporus jarrovi* (14), the release of melatonin, 5MT and 5MIAA from the trout pineal gland were found independent of melatonin deacetylation (15). Although an aryl acylamidase has been shown to be present in the rat pineal (16), attempts to demonstrate deacetylation of melatonin in the rat brain and pineal gland were unsuccessful (17). Therefore, deacetylation of melatonin was not considered to be an important biosynthetic pathway in the pineal gland (3).

Recent studies also suggest that the biosynthesis of 5MIAA (18) and another methoxyindole, 5-methoxy-

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Abbreviations: 5MT, 5-methoxytryptamine; 5MIAA, 5-methoxyindole-3-acetic acid; 5MTL, 5-methoxytryptophol; HIOMT, hydroxyindole-*O*-methyltransferase; GC/EC-NICI-MS, gas chromatography/electron-capture negative ion chemical ionization mass spectrometry.

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tryptohol (5MTL) (19) in the retina might be different from that of the pineal gland in different vertebrates. The species variations may reflect different functions of methoxyindoles in different animals. However, limited studies have been devoted to the occurrence and physiological roles of 5MT and 5MIAA in the retina of different vertebrates. In this communication, we report the identification and measurement of 5MT and 5MIAA in the retina of the rat and quail by gas chromatography / electron-capture negative ion chemical ionization mass spectrometry (GC/EC-NICI-MS). Moreover, retinal melatonin exhibits a diurnal variation. The question of whether 5MT and 5MIAA, the two derivatives of retinal melatonin, also display diurnal rhythms and may possibly be implicated in the photoperiodic response processes of the retina was also investigated.

MATERIALS AND METHODS

Male Sprague-Dawley rats (age 6-7 weeks, average body weight 150 ± 3 g, mean ± SEM, n = 60) and male quails (average body weight 110 ± 4 g, mean ± SEM, n = 16) were adapted for at least 7 days to a 12:12 hours light-dark cycle. Decapitations were performed at either mid-light or under a 25 W red light at mid-dark. The retinas were removed, immediately frozen in liquid nitrogen, and 5MT and 5MIAA was extracted from the samples immediately (mid-light samples) or the next day (mid-dark samples).

The retina samples (n=1 for quail; pooled, n=2-4 for rat) were homogenized in 2 × 1 ml of methanol and mixed with an appropriate amounts of 5-methoxy-[α, α, β, β-$^2H_4$]-tryptamine hydrochloride (d_4-5MT, Sigma Chemical Co., St. Louis, Miss., U.S.A.) and 5-[$^2H_3$]-methoxyindole-3-acetic acid (d_3-5MIAA) internal standards (21). Proteins were removed by centrifugation at 16,000 rpm for 15 minutes, and the dried residue was resuspended in 2 ml of 0.4 M perchloric acid and centrifuged at 16,000 rpm for further removal of proteins. Fatty substances were removed by extraction with purified hexanes (2 × 5 ml). 5MIAA and 5MT in the sample were extracted with 2 × 5 ml of dichloromethane at pH 2.0 - 2.5 and 12.5, respectively. By spiking quail retina samples with radioactive [$^3H_3$]-5-methoxytryptamine and [$^3H_3$]-5-methoxyindole-3-acetic acid prepared in our laboratory (Tsang et al., unpublished results), the % recoveries were found to be 87 ± 2% and 89 ± 2% (mean ± c.v., n=6) for 5MIAA and 5MT, respectively.

The sample derivatization, equipment and procedure for GC/EC-NICI-MS analysis were fully described in two recent reports (20,21). Briefly, 5MT in the retina samples was determined by measuring ion intensity ratios at m/z 321/324 and 342/345 of the 3,3′-spirocyclic pentafluoropropionyl indolenine derivatives of [$^2H_3$]-acetyl-5MT (d_3-Ac-5MT, sample) and d_3-Ac-d_4-5MT (internal standard) at a GC retention time of 5.5 minutes. 5MIAA in the retina samples were determined in separate GC/MS runs by measuring ion intensity ratios at m/z 433/436 and 413/416 of the N-trifluoroacetyl-O-pentafluoropropyl ester derivative of 5MIAA (sample) and d_3-5MIAA (internal standard) at a GC retention time of 7.1 minutes. If the level of 5MT or 5MIAA found at two intensity ratios agreed within ±20%, then the presence of 5MT or 5MIAA in the sample was accepted, and the average value was taken as the final result.

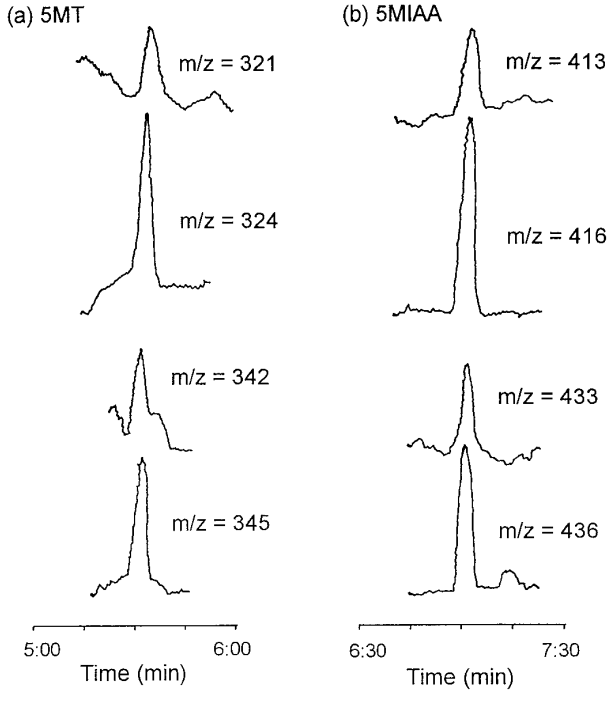

FIG. 1. Typical GC/EC-NICI-MS chromatogram profiles of rat retina (a) 3,3′-spirocyclic pentafluoropropionyl indolenine derivatives of d_3-Ac-5MT (sample) and d_3-Ac-d_4-5MT (internal standard) at m/z 321/324 and m/z 342/345 and (b) N-trifluoroacetyl-O-pentafluoropropionyl ester derivatives of 5MIAA (sample) and d_3-5MIAA at m/z 413/416 and m/z 433/436.

TABLE 1

Level of Endogenous 5MT and 5MIAA in the Retina of the Rat and Quail (pg/Pair of Retinas)

Sample	Time	5MT level (pg/pair of retinas) (mean ± S.E.M.)	5MIAA level (mean ± S.E.M.)
Rat[a]	Mid-light	50 ± 4 (n = 10)*	42 ± 3 (n = 8)*
	Mid-dark	13 ± 1 (n = 10)*	24 ± 2 (n = 8)*
Quail[b]	Mid-light	221 ± 11 (n = 8)*	120 ± 8 (n = 8)*
	Mid-dark	488 ± 28 (n = 8)*	340 ± 24 (n = 8)*

[a] n = number of pooled samples from 2 rats conditioned at mid-light, 4 rats conditioned at mid-dark.

[b] n = number of sample from one quail.

* indicates significant difference between mid-light and mid-dark by ANOVA analysis ($p < 0.05$)

RESULTS

Using the ultra-sensitive and specific GC/EC-NICI-MS technique, we have demonstrated definitively the presence of endogenous 5MT and 5MIAA in the retina of rats and quails (Figure 1). The detection limits achievable on our GC/MS system were 0.15 and 0.50 picogram equivalent of 5MT and 5MIAA standards per injection, respectively, at a signal to noise (S/N) ratio of $\geqslant$2.

Retinal 5MT and 5MIAA were found at femtomole to picomole per pair of retinas levels (Table 1), and are comparable to the melatonin levels reported for rats (22), hamsters (23) and quails (24). In a recent study,

5MT was not detected in the bovine retina by GC/EC-NICI-MS (25). To our knowledge, this is the first report on the retinal levels of 5MT in laboratory animals. The retinal 5MIAA levels in the rat are comparable to that found in the European hamster by Beck and Pévet (23). The present set of GC/EC-NICI-MS data also confirms our previous results on retinal 5MIAA in the quail (18), which were obtained by a less sensitive, electron-impact ionization mass spectrometric technique.

Significant diurnal variations (about 2-3 folds) of retinal 5MT and 5MIAA were observed in the rat and quail. However, the parallel diurnal variations of 5MT and 5MIAA showed inter-species differences. In the rat, high levels of retinal 5MT and 5MIAA were observed at mid-light, whereas high levels of these two methoxyindoles were observed at mid-dark in the quail.

DISCUSSION

The present study is the first report on the circadian rhythms of retinal 5MT and 5MIAA in the rat, with high levels found at mid-light. Our results are consistent with the diurnal variation of 5MIAA found in the hamster retina (23). The diurnal variations observed are opposite to that of retinal melatonin which shows high levels at mid-dark in rats and quails (22,26). In addition, the retinal 5MT and 5MIAA rhythms are similar to those found in the rat pineal gland (21,27 and Tsang et al., unpublished work). This is in line with the suggestion that, in the rat, similar biosynthetic pathways for methoxyindoles are occurring in the retina and pineal. There is no significant HIOMT circadian rhythm in the retina and pineal (12,28,29). However, NAT exhibits a pronounced diurnal rhythm with high activities at night (12). Our results and those reported by others (3,18,19) suggest that the rapid decrease in pineal and retinal NAT activity at the onset of the light period is responsible for the shift of N-acetylation to O-methylation of serotonin to form 5MT, which is subsequently converted to 5MIAA. This may account for the inverse relationship between retinal melatonin and 5MT or 5MIAA levels in the rat. The proposed deacetylation of retinal melatonin to 5MT by aryl acylamidase in lower vertebrates (9,14) is apparently not important in the rat retina.

In the quail, the circadian rhythms of retinal 5MT and 5MIAA are of similar magnitude (about 2-3 folds higher at night) and in-phase with that of retinal melatonin (about 5 folds higher at night) (24,26). A parallel circadian variation of retinal 5MTL has also been reported (19,30). Taken together, the parallel circadian rhythms of retinal melatonin, 5MT, 5MIAA and 5MTL in the quail are highly supportive of the melatonin deacetylation pathway proposed by Besharse and coworkers (9,14), in which 5MT, 5MIAA and 5MTL are the metabolic degradation products of melatonin. Our results also showed that retinal 5MT and 5MIAA levels in the quail were about an order of magnitude (10 folds) higher than those in the rat. These findings are consistent with the reported melatonin and HIOMT activities in the bird retina, which are over 10-folds higher than those in the rat or rabbit (2,29). The HIOMT activity determines the overall conversion efficiencies of serotonin to methoxyindoles and the relative amounts of methoxyindoles found in the retina of different vertebrate species, irrespective of the biosynthetic pathways involved (29).

The presence of measurable amounts of rhythmic 5MT and 5MIAA suggest that they too, like melatonin, could be factors involved in the circadian response processes of the retina. The present study shows that, in the rat, the retinal formation of 5MT and 5MIAA is competitive with that of melatonin while in the quail, their rhythms are likely secondary to that of melatonin synthesis. Whether this has a role in the nocturnal habits of rats and the diurnal activities of quails should be considered. Further studies are needed to explore the inter-species differences and the functional roles of these methoxyindoles in different animals.

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