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Elevated Daytime Melatonin Concentrations in Autism: A Pilot Study

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Concentrations of melatonin in overnight and first-voiding urine samples from 10 people with autism, 15 parents, 1 grandparent, 9 sibs without autism, and 10 healthy, unrelated volunteers, were measured by radioimmunoassay. Those with autism had significantly higher melatonin concentrations in the first voiding samples than controls. Groups did not differ in overnight melatonin concentrations. These preliminary results warrant replication and extension.

Introduction

We previously reported (Ritvo et al., 1988) abnormally low b-wave amplitudes in the electroretinograms (ERGs) of subjects with autism which we subsequently noted to be more marked in early morning than in the late afternoon (unpublished data). This was suggestive of circadian influences on response amplitude. Melatonin production by the pineal gland of the adult is known to show a marked diurnal variation which peaks in the early morning around 2 AM and drops to very low values during the day (Wetterberg, 1978). In the neonatal rat, melatonin production is elevated both day and night and the diurnal pattern seen in the adult develops over the first 12 days of life in accordance with maturation of noradrenergic terminals on pinealocytes (Yuwiler et al., 1977). Melatonin has recently been reported (Dubocovich 1983) to inhibit calcium dependent dopamine release from amacrine cells and amacrine cells interact with the retinal bipolar cells which generate the b-wave (Duke-Elder, 1979). Based on these reports we hypothesized that perhaps as a reflection of the perversive developmental delays in autism, melatonin production in autism might persist into the early morning, thereby inhibiting dopamine release from amacrine cells and accounting for the reduced b-wave amplitude found in these subjects.

In this paper we examined melatonin concentration in overnight and first voiding urine to test this proposition. Although the bulk of melatonin produced by the pineal is 6-hydroxylated in liver and appears in the urine as sulfate or glucuronide conjugates of 6-hydroxymelatonin, free melatonin was measured in this study so as to permit comparison with urinary melatonin values in the literature and also because the conjugates reflects the enzymatic activities of at least two liver enzymes as well as of melatonin concentration, and the sensitivity of these enzymes to environmental conditions has not yet been fully defined. In addition, melatonin freely passes into the kidney tubule without resorption thereby reflecting blood melatonin concentration (Sääf et al., 1980). It thereby provides a picture of integrated blood melatonin concentration over the period of urine collection and overnight urine concentration strongly correlates ($r = 0.8$) with 2 AM serum melatonin (Almay et al., 1987). Because of their more polar nature, it is not clear if the same is true for 6-hydroxymelatonin conjugates.

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Methods

Subjects

Ten people with autism, 15 parents, 1 grandparent, 9 siblings, and 10 unrelated healthy comparison subjects without autism drawn from 4 families were studied. Diagnoses were made as previously described (Ritvo et al., 1989). All patients met DSM-III and DSM-III-R criteria for full syndrome (299.0). The mean IQ for the subjects with autism was 78 and only one had an IQ below 70. Only one subject, with an IQ of 80, had a history of possible seizures.

Assay

Urinary melatonin was assayed by radioimmunoassay using a commercially available kit (K-Lab, Walnut Creek, California).

Samples

Two samples were obtained for each subject. Subjects were instructed to empty the bladder before retiring between 10 PM to 11 PM and to empty the bladder and collect the first voiding the next morning at approximately 7 AM to 8 AM. This sample, which included the including the 2 AM peak, is identified here as the "overnight" sample. The second sample was collected at the next voiding, between 10 AM to 11 AM, and is designated here as the "day" sample.

Values for total urinary excretion were not obtained in this study because of logistic difficulties in collecting and measuring total urine volume.

Statistics

Data was analyzed by a 1-way Analysis of Variance using BMDP program 7D (BMDP Statistical Software Inc. Los Angeles, CA 90025). Bonferroni and Newman-Keuls tests, which take account of multiple comparisons, were used to evaluate group differences. Since melatonin values are not normally distributed, statistical computations were also carried out using log (melatonin), which is normally distributed, to verify the statistical comparisons. BMDP program 2V was used for Analysis of Covariance using age as covariate and program 6D was used to examine the correlation between melatonin and age.

Results

As shown in Table 1, groups did not differ in overnight melatonin ($F = 0.5$; $Df = 3,41$; $p = 0.7$) but did in daytime melatonin ($F = 3.9$; $Df = 3,41$; $p = 0.016$) and in the ratio of daytime to overnight melatonin ($F = 6.2$; $Df = 3,41$; $p = 0.001$). Multiple range tests showed that subjects with autism differed from normals at $p<0.01$ in daytime melatonin concentration and that subjects with autism and their parents differed from normals the ratio of daytime-to-overnight concentrations ($p<0.05$). As might be expected, parents were significantly older than their offspring. Controls were signifi-

Table 1. Population values for age and urinary melatonin.

	Normal	Autism	Sibs	Parents
Number	10	10	9	16
Age (yrs)	35 ± 6	18 ± 2	20 ± 4	50 ± 3
Mel. Night (μM)	253 ± 40	276 ± 49	300 ± 39	238 ± 34
Mel. Day (μM)	79 ± 6	.192 ± 33	137 ± 25	147 ± 18
Ratio (%)	35 ± 4	73 ±5	48 ± 7	74 ± 9

Statistical results from ANOVA and Bonferroni and Tukey analysis. Groups significantly differed in age ($F = 18$; $Df = 3,41$; $p = 0.0001$). Parents of subjects with autism were significantly older than the normal controls $p = 0.01$ who were older than the children with autism or their sibs. Nighttime melatonin was the same for all groups $F = 0.5$; $Df = 3,41$;$p = 0.7$. Groups differed in daytime melatonin ($F = 3.9$;$Df = 3,41$;$p = 0.016$). Values for subjects with autism were significantly higher than values for normal controls ($p<0.01$). Groups also significantly differed in the ratio of daytime-to-overnight melatonin ($F = 6.2$, $Df = 3,41$; $p = 0.001$). Both children with autism and their parents differed from normal at $p<0.05$.

cantly older than both people with autism and their siblings.

Group differences were even sharper when log (melatonin) was used for computations. Groups differed in daytime melatonin ($F = 5.7$; $Df = 3,41$; $p = 0.0023$) and in the ratio of daytime-to-overnight melatonin ($F = 6.6$; $Df = 3,41$; $p<0.001$) and in both cases subjects with autism and their parents differ from normals ($p<0.05$).

As in other studies, age correlated with overnight melatonin concentration ($R = 0.36$ for males, $R = 0.26$ for females and $R = 0.26$ for both combined, although the correlation failed to reach statistical significance ($p = 0.08$, $N = 45$). Age was unrelated to morning melatonin for either sex or for both sexes combined. Despite this the data were reanalyzed using an Analysis of Covariance with age as covariate to assure that age did not account for the group differences. Again, however, the groups differed in daytime-melatonin ($F = 3.6$; Df 3,1,40; $p = 0.022$) and in the daytime/overnight ratio ($F = 6.5$; $Df = 3,1,40$; $p = 0.0011$).

Discussion

Normally, melatonin production in man is elevated at night, peaks at 2 AM and falls towards zero during daylight (Wetterberg et al., 1978). Although the number of subjects in this study is too small to permit a definitive assertion of abnormal melatonin production accompanying autism, the results are provocative. Our populations did not differ in nocturnal melatonin production, but melatonin production by people with autism, some of their parents and some of their unaffected sibs appeared to persist into the daylight hours. Extended daylight secretion of melatonin is most unusual and could account for the decrease b wave amplitudes observed in the morning electroretinograms of some subjects with autism.

Groups differed in age and melatonin production declines with age. However, factoring out the population differences in age did not affect the population differences in daytime melatonin excretion or the ratio between daytime and overnight melatonin.

Familial factors may contribute to the present findings. While daytime melatonin is highest for people with autism, values for parents are nearly as high and those for siblings are intermediate. Such familial factors may be linked to the incidence of autism itself or may be secondary to effects on melatonin production, metabolism, or regulation. If larger studies corroborate the present findings, pursuit of the mechanism of the familial relationship could be of considerable interest.

Although most of the melatonin excreted in urine is in the form of the 6-sulfatoxy derivative (Young et al., 1985), we measured melatonin itself since it is passively absorbed through the kidney tubule in equilibrium with blood melatonin *concentration* (Sääf et al., 1980) and correlates well with blood melatonin (Lang et al., 1981). We did not compute total melatonin excretion or mean excretion rate in this study because of difficulties in getting complete urine collections and compliance with measurement procedures. It will be interesting, in future studies, to examine the correlation between melatonin content and concentration, the parent compound and its catabolite, and the utility of the catabolite in differentiating people with autism and control.

Although provocative and in conformity with the initiating hypothesis, methodological problems limit the generality of the results. As mentioned, population sizes are marginal for statistical analysis. The available normal control population is not adequately matched with the experimental populations with regard to age, sleep patterns, or activity level. Because of its outpatient character, collection and identification of samples was uncontrolled and assumed parental adherence to instruction. Finally, light exposure during the night was uncontrolled as were bedtimes. Clearly this pilot study needs replication with a larger population under more controlled conditions than are currently available to us.

Résumé

Les concentrations de mélatonine sur l'ensemble de la nuit et la première émission d'urine provenant d'échantillon de 10 sujets atteints d'autisme, 15 parents, 1 grandparent, 9 membres de la fratrie sans autisme et 10 volontaires sains sans relation avec les patients, furent mesurés par une technique de radio immuno-essai. Les patients avec autisme ont des concentrations de mélatonine significativement plus élevées dans les échantillons de première émission que les contrôles. Les

groupes ne diffèrent pas dans les concentrations de mélatonine durant la nuit. Ces résultats préliminaires demandent à être répétés et élargis.

Zusammenfassung

Die Urinkonzentrationen von Melatonin wurden von 10 Personen mit Autismus, 15 Elternteilen, einem Großelternteil, 9 Geschwistern ohne Autismus und 10 gesunden, nicht verwandten freiwilligen Kontrollpersonen im Radioimmunoassay bestimmt. Die Urinproben wurden über Nacht und bei erster Blasenentleerung gewonnen.

Personen mit Autismus hatten signifikant höhere Melatoninkonzentrationen in den Urinproben, die nach erster Blasenentleerung gewonnen wurden. Die Gruppen unterschieden sich nicht im Hinblick auf die Konzentrationen in den Urinproben, die bei Nacht gewonnen worden waren. Diese vorläufigen Ergebnisse sollten repliziert und ausgedehnt werden.

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