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Acute treatment with desipramine stimulates melatonin and 6-sulphatoxy melatonin production in man

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1 Acute administration of the antidepressant drug desipramine (DMI) in man, increased evening melatonin secretion, which reached peak plasma levels 2–4 h earlier than after placebo administration.

2 The increase at set time points 21.00 h–22.00 h was directly proportional to an individual's integrated night-time secretion of melatonin.

3 We have shown that this stimulation was not an effect of DMI inhibition on the hepatic metabolism of melatonin to 6-sulphatoxy melatonin (aMT6s), indeed aMT6s is in itself a good index of the evening melatonin rise.

4 The stimulation of early evening melatonin by DMI might be exploited as a simple pineal function test.

Keywords desipramine melatonin

Introduction

The rhythmic production of melatonin from the pineal gland at night, in a normal light-dark cycle, is known to depend upon the integrity of the sympathetic innervation of the gland (Wurtman *et al.*, 1964), the suprachiasmatic (SCN) and paraventricular nuclei (Moore & Klein, 1974; Klein *et al.*, 1983a), the retino-hypothalamic projection, and the eyes, at least in the rat (for a review, Klein, 1979). At present it is considered that a 'central rhythm generator' situated in the SCN generates an endogenous rhythm in melatonin production which is entrained to 24 h by the light dark cycle. The final common pathway controlling melatonin synthesis lies in the sympathetic innervation of the gland terminating at the β_1-adrenoceptor (in the rat, Klein, 1979). Rhythmic neural input is coupled to variable receptor sensitivity: β-adrenoceptor sensitivity increases to a maximum at the end of the light-phase (Romero & Axelrod, 1974): noradrenergic stimulation in the dark phase leads, in a sequence, to an increase in cAMP, stimulation of serotonin-*N*-acetyl transferase activity and a resulting increase in melatonin synthesis and secretion (Wilkinson *et al.*, 1977; Klein, 1979). Recently α-adrenoceptors have also been implicated in the stimulation of melatonin synthesis (Klein *et al.*, 1983b).

As a result of these mechanisms controlling melatonin production, and also the fact that it appears to be secreted directly into the peripheral circulation (Rollag *et al.*, 1978), its secretion has provided a marker in psychiatric studies for rhythm disturbances, the effects of light, and for the study of β-adrenoceptor function (Beck-Friis *et al.*, 1974; Lewy *et al.*, 1978; Thompson *et al.*, 1983). There is good evidence that β_1-adrenoceptors mediate human melatonin secretion (Cowen *et al.*, 1983; Arendt *et al.*, 1985).

Clinical studies (Thompson *et al.*, 1985) have shown that chronic administration of the tricyclic antidepressant desipramine (DMI), resulted in increased melatonin levels in depressed patients but no increase in normal volunteers. Acute administration of oxaprotiline, a specific noradrenaline uptake inhibitor, and DMI increased

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plasma melatonin in normal volunteers over a 24 h period (Checkley *et al.*, 1985). Since it is known that tricyclic drugs modify the metabolism of certain steroids (Morselli, 1979) and inhibit the metabolism of amphetamine (Dingell & Bass, 1969) it is important to establish that the stimulation of melatonin is not due to an inhibition of its metabolism by DMI. Moreover, the acute administration of DMI may be of use as a test of pineal function since to date there is no clinically acceptable and established stimulatory test of melatonin secretion. We wished to establish whether the melatonin response to DMI was related to an individual's capacity for endogenous secretion.

We report two inter-related studies (a and b): (a) We describe the stimulatory effect of a single oral dose of DMI upon plasma melatonin in normal subjects and the relationship of this response to the subjects' endogenous night-time plasma melatonin secretion. The resulting plasma and urinary levels of a major metabolite of melatonin, shown in the rat (Kveder & McIsaac, 1961) and in man (Jones *et al.*, 1969), to be 6-sulphatoxy melatonin (aMT6s) are also presented. (b) We report upon the effects of DMI administration during the day, upon the metabolism of a small dose (200 μg) of exogenous melatonin.

Methods

Study A

Subjects The study was performed on eight healthy male volunteers aged 20–35 years recruited from staff at the Maudsley Hospital, London. All subjects were drug free and within 10% of their ideal body weight. Ethical approval for this study was obtained from the Maudsley Hospital Ethics Committee.

Treatment The administration of DMI was timed to ensure maximum plasma concentrations 2 h before the onset of nocturnal melatonin secretion. Each volunteer received on one occasion DMI 100 mg orally at 16.00 h and on the other a matching placebo. The study design was double blind and the order of drug administration was balanced. The two experiments on each subject were separated by 1 week. The study was conducted during July and August 1984 and on each occasion lights were out between 24.00 h and 07.00 h.

Sampling An intravenous cannula was inserted at 15.00 h and kept patent overnight by a slow infusion of heparinised saline using a battery operated pump. Plasma samples were taken at hourly intervals from 15.00 h until 09.00 h on the next day. Overnight urine was collected between 22.00 h and 07.00 h on each occasion and samples were stored at −20° C. Samples were assayed for DMI and melatonin (plasma) and aMT6s (plasma and urine).

Study B

Subjects Four healthy volunteers from the University of Surrey, three male and one female, aged 22–27 years participated. All subjects were drug free. Ethical approval for this study was obtained from St Lukes Hospital, Guildford, Ethics Committee.

Treatment Each volunteer received the following oral treatments: (a) DMI 100 mg at 09.00 h followed by melatonin 200 μg at 13.00 h; (b) matching (DMI) placebo tablets at 09.00 h followed by melatonin 200 μg at 13.00 h; (c) DMI 100 mg at 09.00 h followed by matching (melatonin) placebo at 13.00 h.

DMI (Pertofran® Geigy) 4 × 25 mg tablets or matched placebo tablets were administered in a soft centred chocolate. Melatonin, 200 μg was dissolved in 5 ml 2% ethanol and sunflower oil (for pharmacokinetic comparison with other preparations see Aldhous *et al.*, 1985). Melatonin and placebo (vehicle only) were ingested with a glass of water.

The study design was double-blind and the minimum interval between treatments was 1 week. The study was conducted under normal laboratory lighting and subjects ate a standard breakfast and lunch on all occasions.

Sampling Six hourly urine samples were collected sequentially for 24 h from each subject before treatment.

An intravenous cannula was inserted at 12.15 h on each treatment occasion and plasma samples were collected at −0.25, 0, 0.25, 0.5, 0.75, 1, 2, 3 and 4 h after administration of melatonin or placebo. Three urine samples were collected sequentially until 17.00 h the following day from 13.00 h–17.00 h, 17.00 h–12.00 h and 12.00 h–17.00 h. All samples were stored at −20° C. Samples were assayed for melatonin (plasma) and aMT6s (plasma and urine).

Assays

Plasma DMI was estimated using gas liquid chromatography with nitrogen detection (Braithwaite, 1979).

Plasma samples were assayed for melatonin by radioimmunoassay (Fraser *et al.*, 1983) using a tritiated melatonin tracer (NEN, Ltd) and antiserum 704/189 (Guildhay Antisera). The sensitivity ranged from 5 pg ml^{-1} to 10 pg ml^{-1} of plasma and the inter assay coefficients of variation were 12.1% and 8.5% at 62 pg ml^{-1} and 28 pg ml^{-1} respectively. Intra assay coefficients of variation were 2.6% and 10% at 151 pg ml^{-1} and 54 pg ml^{-1} respectively. The confidence limit for the lower limit of detection of melatonin was set at 10 pg ml^{-1} for all calculations.

The plasma and urine samples were also assayed for aMT6s by radioimmunoassay (Arendt *et al.*, 1985). Coefficients of variation inter assay were 8.8% and 10.6% at 40 pg ml^{-1} and 61 pg ml^{-1} (plasma) and 12.9% and 9.9% at 83 pg ml^{-1} and 32 pg ml^{-1} (urine) respectively. Intra assay coefficients of variation were 3.3% and 3.4% at 28 pg ml^{-1} and 74 pg ml^{-1} (plasma) and 3.3% and 4.1% at 4.8 pg ml^{-1} and 113 pg ml^{-1} (urine) respectively. Assay sensitivity of aMT6s was 13 pg ml^{-1} plasma and 0.65 ng ml^{-1} urine.

Analysis of data

Study A Total melatonin secretion after placebo was calculated by area under the night-time profile from 15.00 h to 09.00 h (area under the curve, AUC) using a computer programme 'Stripe' (Johnston & Woollard, 1982). The statistical significance of the effect of DMI on plasma concentrations of melatonin at each time point was determined using two tailed paired *t*-tests. To compare the melatonin response to DMI with the individual night-time endogenous secretion, linear regression analysis was performed.

Study B The statistical significance of differences in urinary aMT6s levels between treatments were determined using two tailed paired *t*-tests.

Results

Study A

Mean plasma DMI levels reached a maximum (24 ng l^{-1}) at 19.00 h, 3 h after administration and remained above 20 ng l^{-1} throughout the period of sampling.

Figure 1 shows mean hourly plasma melatonin concentrations for eight normal subjects 1 h before and 17 h after 100 mg DMI or placebo administration. The large variation in plasma melatonin levels between individuals (but intra-individual consistency) has been reported (Arendt, 1978). There was a significant increase in plasma melatonin concentrations at 21.00, 22.00, 23.00 h ($P < 0.05$) and at 24.00 h ($P < 0.01$) after DMI administration compared with placebo. Inspection of the data suggests that DMI induced a 'phase advance' of melatonin secretion. However no significant differences between treatments were found during the declining phase of the rhythm. It should be noted that the relatively high melatonin levels observed at 09.00 h and indeed over the whole profile are due largely to two individuals whose night-time secretion peaked at 188 pg ml^{-1} and 111 pg ml^{-1} respectively. Total melatonin secretion after DMI (AUC) was not significantly different from total secretion after placebo.

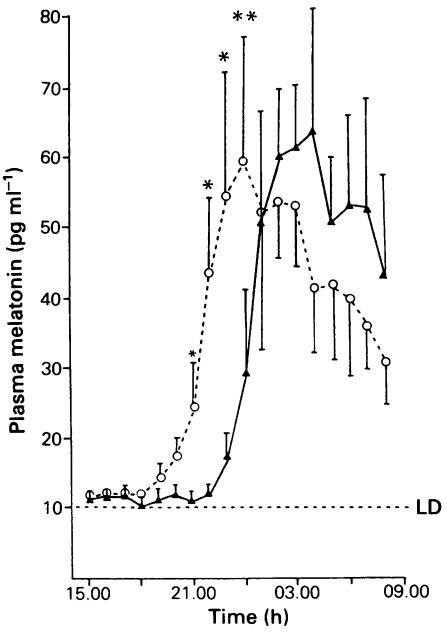

Figure 1 Mean hourly plasma melatonin concentration (± s.e. mean) in eight normal subjects after desipramine 100 mg (○) or after placebo (▲), at 16.00 h. $*P < 0.05$, $**P < 0.01$.

DMI treatment clearly increased plasma melatonin 2 h before the endogenous night-time rise, achieving a peak height 2–4 h earlier than the endogenous peak. We compared each subject's response with their individual capacity to secrete melatonin. The size of the individual increase in plasma melatonin after DMI expressed as the ratio:

$$\frac{\text{Plasma melatonin pg ml}^{-1}\text{ after DMI}}{\text{Plasma melatonin pg ml}^{-1}\text{ after placebo}}$$

at the 22.00 h time point is proportional to an individual's total melatonin secretion (AUC) after placebo ($P < 0.001$, $r = 0.972$). This was also significant at 21.00 h ($P < 0.001$, $r = 0.934$) but not at 23.00 h or 24.00 h.

The effect of DMI on the production of aMT6s is shown in Figure 2 where mean hourly plasma levels of aMT6s are shown 1 h before and for 17 h after DMI administration. Large variations in endogenous melatonin production and metabolism produce large standard errors of the mean. However plasma aMT6s levels after DMI were significantly increased at 23.00 h and 24.00 h ($P < 0.05$) compared to placebo, indicating that the metabolite is in itself a good index of the early evening increase. Total aMT6s excretion after DMI (AUC) was not significantly different from total excretion after placebo. Since melatonin and aMT6s profiles closely mirror each other the results suggest that DMI has no major effect upon the metabolism of melatonin. Urinary aMT6s results (Figure 3) support the plasma data: DMI had no overall effect upon urinary aMT6s excretion from 22.00 h–07.00 h. Whether it modifies morning aMT6s excretion (i.e. after 07.00 h) was not tested.

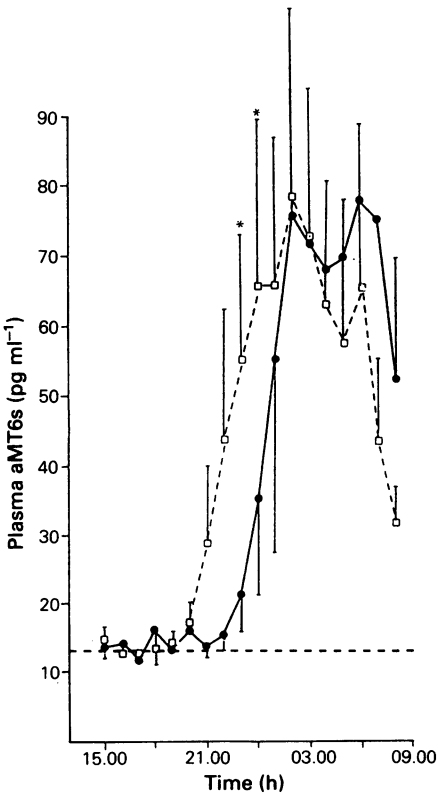

Figure 2 Mean hourly plasma aMT6s concentration (± s.e. mean) in eight normal subjects after desipramine 100 mg (□), or after placebo (●), at 16.00 h. *$P < 0.05$.

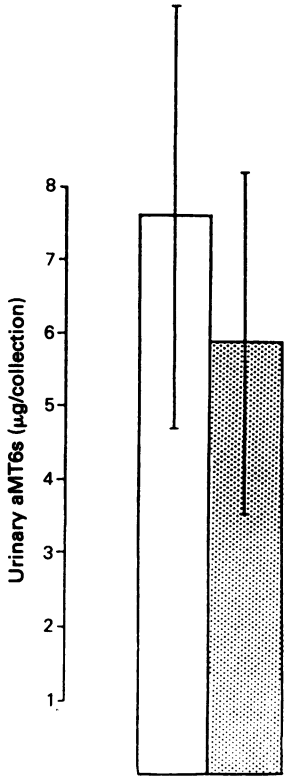

Figure 3 Mean overnight (22.00 h–07.00 h) urinary aMT6s excretion (± s.e. mean) after desipramine 100 mg (■) or after placebo (□) at 16.00 h.

Study B

To assess the effect of DMI on the metabolism of exogenous melatonin, plasma melatonin and plasma and urinary aMT6s concentrations were measured after ingestion of melatonin following either DMI or placebo. Results from the administration of DMI alone at 09.00 h showed no significant stimulatory effect of the drug on either daytime endogenous plasma melatonin, or on aMT6s levels (not shown). An overall interpretation of plasma results after ingestion of melatonin was not attempted, since the absorption of melatonin in two subjects following DMI administration was delayed, reaching peak heights in the plasma 2–3 h later than those following placebo administration (not shown). The results from the urine collections provide a clearer picture (Figure 4).

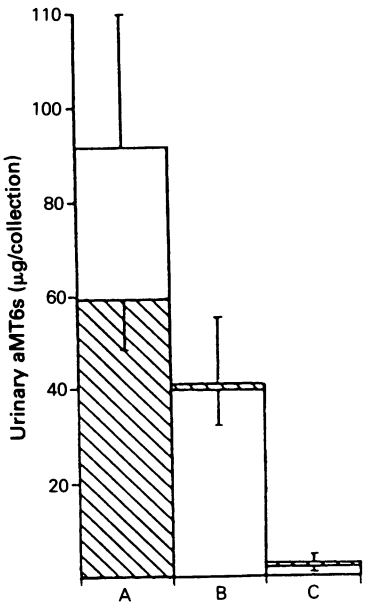

Figure 4 Mean exogenous aMT6s excretion (± s.e. mean) following 200 μg melatonin at 13.00 h, preceded by either DMI (□) or placebo (▨) at 09.00 h. Sequential sample collection: A, 13.00 h–17.00 h; B, 17.00 h–12.00 h; C, 12.00 h–17.00 h.

The contribution of exogenous aMT6s to measured values was assessed in each individual by subtracting: (a) endogenous aMT6s excretion (before treatment) from total aMT6s excretion following placebo (DMI) and melatonin, and (b) aMT6s excretion (after DMI only) from total aMT6s excretion following DMI and melatonin. The majority of exogenous melatonin was metabolised and excreted within the first two collection periods. No significant differences were found in excreted exogenous aMT6s following DMI or placebo. Clearly the metabolism of melatonin was not inhibited by DMI. Moreover, there was an overall tendency towards higher metabolite levels in the presence of DMI, but this was not significant. DMI given in the morning did not significantly alter endogenous urinary aMT6s levels over a 24 h period although there was a trend towards increased output of aMT6s after DMI, particularly in two volunteers.

Discussion

These results show that an acute dose of DMI at 16.00 h increases plasma melatonin in the early evening. Peak melatonin levels were reached 2–4 h earlier after DMI than after placebo administration. Total melatonin secretion (AUC) was not increased after DMI in contrast to previous observations (Checkley *et al.*, 1985), but in these previous experiments the total dose of DMI was very much greater. Although melatonin (Kveder & McIsaac, 1961) and DMI (Potter *et al.*, 1974) are metabolised by hepatic hydroxylation, it is unlikely that the increase is due to DMI inhibition of melatonin metabolism since there was no significant overall inhibitory effect of acute DMI treatment on plasma or urinary aMT6s levels. Indeed, measurement of plasma aMT6s levels proved to be a reasonably good index of the early evening increase. Furthermore, DMI had no significant effect, in a small number of subjects, upon the metabolism of an exogenous dose of melatonin to aMT6s. It is possible that DMI affected melatonin metabolism other than to aMT6s. This is unlikely as aMT6s appears to be the major metabolite of melatonin in man (Jones *et al.*, 1969).

It is most likely that DMI increases plasma melatonin by effectively increasing synaptic noradrenaline as a result of inhibition of noradrenaline reuptake into pineal sympathetic neurones. Pineal β-adrenoceptor sensitivity exhibits a circadian rhythm (Romero *et al.*, 1975) with maximum receptor sensitivity at the end of daylight. Therefore the increase in plasma melatonin was probably dependent upon achieving maximum DMI levels at around 21.00 h. The DMI induced increase in plasma melatonin at 21.00 h–22.00 h was proportional to the individual's integrated endogenous secretion. The lack of a significant correlation at later time points is difficult to explain. Either the functional capacity of the pineal to secrete melatonin has reached a maximum or down regulation at pineal β-adrenoceptors has occurred due to increased exposure to noradrenaline at earlier time points. Alternatively the results (Figure 1) may be interpreted as a phase shifting effect of DMI administration advancing the timing of the endogenous rhythm of melatonin. It seems unlikely that an acute dose of DMI would have such an effect particularly since chronic antidepressant administration in animals is reported not to advance but to delay the timing of circadian rhythms (Wirz-Justice *et al.*, 1980). Chronic DMI administration in depressed patients does not alter the timing of melatonin secretion (Thompson *et al.*, 1983).

The measurement of early evening plasma melatonin following acute DMI administration may provide a suitable pineal function test. Clearly this study would have to be extended to larger numbers of subjects to confirm the consistency and reliability of the response. Other authors have reported stimulation of human

melatonin secretion by various means such as administration of destyrosyl-γ-endorphin (Claustrat *et al.*, 1981), ritodrine, a β_2-adrenoceptor agonist (Desir *et al.*, 1983) and heavy exercise (Carr *et al.*, 1981). In none of these reports is the relationship of the response to endogenous secretion known.

In conclusion, acute DMI (100 mg) administered orally at 16.00 h stimulates melatonin secretion during the evening, 5–8 h after the dose. Whether this effect of noradrenaline uptake blockade upon melatonin is exclusively pineal or whether there is also a central component remains a matter for speculation.

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