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Plasma melatonin during desmethylimipramine treatment: evidence for changes in noradrenergic transmission

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1 Plasma melatonin was used to determine overall β-adrenergic transmision through pineal neuro-effector junctions during desmethylimipramine (DMI) treatment in 10 normal subjects.

2 Changes in plasma melatonin indicated that an initial increase in noradrenaline (NA) transmission produced by DMI was counteracted by adaptive changes which restored transmission to normal by the third week of treatment. A 'rebound' increase in NA transmission was seen on DMI withdrawal.

3 The results suggest that the adaptive changes which occur in NA synapses during DMI treatment do not, as has been proposed, decrease NA transmission below normal levels, but instead restore homeostasis in the presence of the drug.

Keywords desmethylimipramine pineal gland melatonin β-adrenoceptors

Introduction

Acute administration of tricyclic antidepressants (TCAs) enhances noradrenergic transmission through blockade of neuronal uptake of noradrenaline (NA) (Iversen & MacKay, 1979). The antidepressant effect of TCAs has often been attributed to this property (Schildkraut, 1965), but in depressed patients TCA treatment may be required for 2 weeks or more before a clear antidepressant effect is seen (Oswald *et al.*, 1972). Accordingly, recent animal studies have assessed the effect on brain NA function of repeated daily administration of TCAs. Particular attention has been directed to changes in post-synaptic β-adrenoceptors since repeated administration of nearly all antidepressant treatments, including TCAs (Banerjee *et al.*, 1977; Sellinger-Barnett *et al.*, 1980), electroconvulsive shock (Pandey *et al.*, 1979a) and monoamine oxidase inhibitors (Sellinger-Barnett *et al.*, 1980), reduces the density of specific β-adrenoceptor binding and/or the responsiveness of the associated NA-sensitive adenylate cyclase (Vetulani & Sulser, 1975; Sulser, 1979) in various brain regions. The consistency of these changes has led to the proposal that decreased β-adrenoceptor synaptic function is responsible for the therapeutic effect of antidepressant treatment (Sulser, 1979).

Because of the findings from animal studies it would clearly be of interest to ascertain the effects of the clinical administration of antidepressant drugs on β-adrenergic synapses in man. We have approached this problem by using the production of the pineal hormone, melatonin, as an index of β-adrenergic function. In both rat (Axelrod, 1974) and man (Cowen *et al.*, 1983a) the production of melatonin is dependent upon the activity in the pineal gland of junctions between NA nerve terminals and β-adrenoceptors on pinealocytes. This activity follows a marked circadian pattern with the bulk of β-adrenergic transmission and melatonin synthesis occurring at night (Axelrod, 1974). Thus, in man plasma melatonin levels are

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elevated at night and, with conventional radioimmunoassays (RIA), generally undetectable in the daytime (Arendt *et al.*, 1982; Fraser *et al.*, 1983a). Animal studies have shown that plasma melatonin levels correlate well with pineal melatonin synthesis (Wilkinson *et al.*, 1977).

We and others have demonstrated that repeated administration of the TCA desmethylimipramine (DMI) to rats down-regulates β-adrenoceptors in the pineal in the same way as β-adrenoceptors in the cerebral cortex (Cowen *et al.*, 1983b; Heydorn *et al.*, 1982; Friedman *et al.*, 1984). In addition, the synthesis of melatonin by the pineal in response to an exogenous β-adrenoceptor agonist is attenuated following repeated DMI treatment (Cowen *et al.*, 1983b; Heydorn *et al.*, 1982), suggesting a lowered functional response of pineal β-adrenoceptors.

The increase in pineal and plasma melatonin at night is dependent on the physiological release of NA from prejunctional NA terminals in the pineal gland (Axelrod, 1974; Cowen *et al.*, 1983a,b). This increase therefore reflects the overall transmission through prejunctional NA neurones and pineal β-adrenoceptors and can be used to determine the net effect on pineal β-adrenergic transmission of repeated antidepressant administration.

In the present study we have investigated the effect of a clinical course of DMI treatment on plasma melatonin concentrations in normal volunteers to assess the effect of this TCA on pineal β-adrenergic transmission. We decided to use normal volunteers since depressive illness itself may affect both β-adrenoceptor function (Pandey *et al.*, 1979b) and the synthesis of melatonin by the pineal gland (Wetterberg *et al.*, 1981), and our aim in this investigation was to study the effect of TCA treatment alone.

Methods

Subjects

Ten healthy male subjects (mean age 31.6 years; range 26–36) gave informed consent to the study which was approved by the Oxfordshire Area Health Authority Psychiatric Ethics Committee. They were free of psychiatric and physical illness and had taken no psychotropic medication during the previous 6 months. The subjects took DMI twice daily starting at a dose of 75 mg at 22.00 h and increasing by 25 mg each subsequent morning up to 150 mg if this could be tolerated without troublesome side effects. Eight subjects took 150 mg and 2 each 125 mg (mean dose 2.1 mg/kg; range 1.6–2.6). The DMI was continued at this dose for a further 14 days and then discontinued after the final 22.00 h dose. The total treatment period was thus 19 days.

Plasma sampling

Subjects were tested over 24 h in a research unit. They were kept in artificial light except for the sleep period which was from 00.30 h to 08.00 h when all lights were extinguished. An indwelling venous cannula was inserted at 10.00 h and blood samples taken each hour until 10.00 h the following day. This sampling procedure was carried out on two occasions, 3 days before starting DMI and again on the final day of treatment. In addition, a single blood sample was taken at 24.00 h at the subject's home after 1, 4 and 7 days' DMI treatment and 1, 2, 3 and 7 days after DMI withdrawal. The subjects were awake and the lighting conditions were similar to those in the research unit.

Melatonin assay

Plasma was separated from blood samples by centrifugation and stored at −20°C until assay. Plasma melatonin was measured by a previously described RIA (Fraser *et al.*, 1983a) which correlates well with gas chromatography mass spectrometry measurement (Fraser *et al.*, 1983b). All the daytime and all the night-time samples of each subject were measured in the same assays. Detection limit of the assay was 11 pg/ml and samples with a concentration less than this were allotted a value of 10 pgm. Average intra- and inter-assay variation over the range of the standard curve were 9.1% and 11.5% respectively.

Measurement of DMI

Plasma DMI concentrations were measured in the midnight plasma samples. An adaptation of a previously described gas-liquid chromatography technique was used (Braithwaite, 1979), employing a Perkin-Elmer F.33 gas chromatograph fitted with a nitrogen/phosphorus detector. The detector was set to the nitrogen mode. Each subject's samples were measured in the same assay. Average inter-assay variation was 10.8% over the range 0–200 ng/ml of DMI in plasma.

Statistics

Differences in plasma melatonin concentration were analysed using a one-way or two-way analysis of variance with repeated measures,

followed by Fisher's test of least significance difference (LSD) where appropriate. Changes in plasma DMI concentration were similarly analysed. Melatonin secretion was also measured as area under the curve using Simpson's rule. A paired *t*-test (two-tailed) was used for this comparison. Correlations were performed by Pearson's method.

Results

The DMI was generally well tolerated at the chosen dose though all subjects had a dry mouth. Six subjects woke earlier than usual while taking DMI but no effect on mood was reported. No significant withdrawal symptoms were seen.

During the 24 h sampling period prior to DMI administration, plasma melatonin levels were elevated at night but, except for one subject, undetectable in the daytime. In the second 24 h sampling on day 19 of DMI treatment the same overall profile of melatonin secretion was apparent but an additional subject now had detectable daytime plasma melatonin. The analysis of variance showed a significant increase in plasma melatonin at night on both sampling occasions ($P < 0.005$), but 19 days' DMI treatment did not change plasma melatonin levels significantly from pretreatment values (Figure 1). Similarly, there was no significant difference in melatonin secretion measured as area under the curve between 20.00 and 09.00 h (paired *t*-test). There was a tendency for the peak melatonin concentration to occur earlier while subjects were on DMI treatment (Figure 1) but this was not significant (paired *t*-test).

The series of single midnight plasma melatonin samples showed a consistent and highly significant ($P < 0.005$, one-way analysis of variance) pattern of change. Two hours after the first dose of DMI, plasma melatonin at midnight was elevated over baseline levels (Figure 2). This increase was maintained as the dose of DMI was increased (Figure 2). However, by day 19 of treatment midnight plasma melatonin levels had fallen compared to those on day 4 and day 7 and were not now significantly higher than baseline (Figure 2). Midnight melatonin was once again increased 1 day after DMI withdrawal but had returned to baseline values 7 days later (Figure 2).

Midnight melatonin concentration during the two 24 h sampling periods correlated well with melatonin secretion calculated as area under the curve ($r = 0.88$, $P< 0.001$), confirming an earlier report that single midnight melatonin samples give a valid measure of 'total' daily melatonin secretion (Arendt *et al.*, 1982). There was similarly a good correlation ($r = 0.88$, $P < 0.001$) between change in midnight melatonin and change in area under the curve of melatonin secretion of individual subjects when the first and second 24 h sampling periods were compared.

Plasma DMI levels showed the expected elevation as the dose of drug was increased

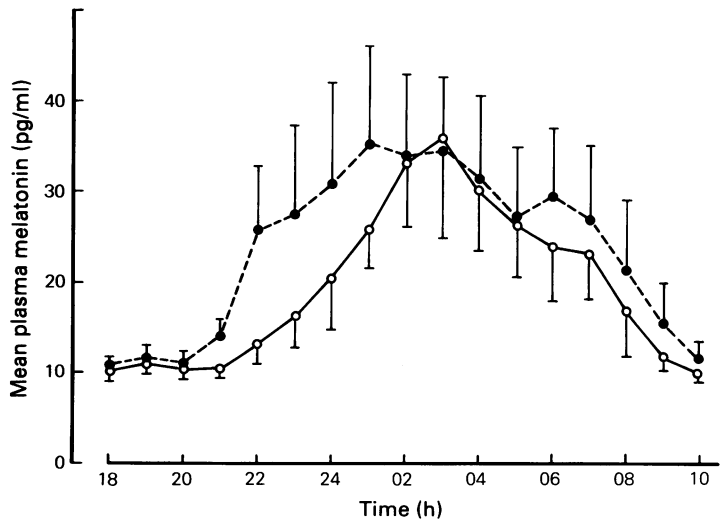

Figure 1 Mean (± s. e. mean) plasma melatonin concentration between 18.00 and 10.00 h in 10 normal subjects tested prior to DMI (solid line) and on the 19th day of treatment (broken line). No significant difference between the two sampling days (analysis of variance).

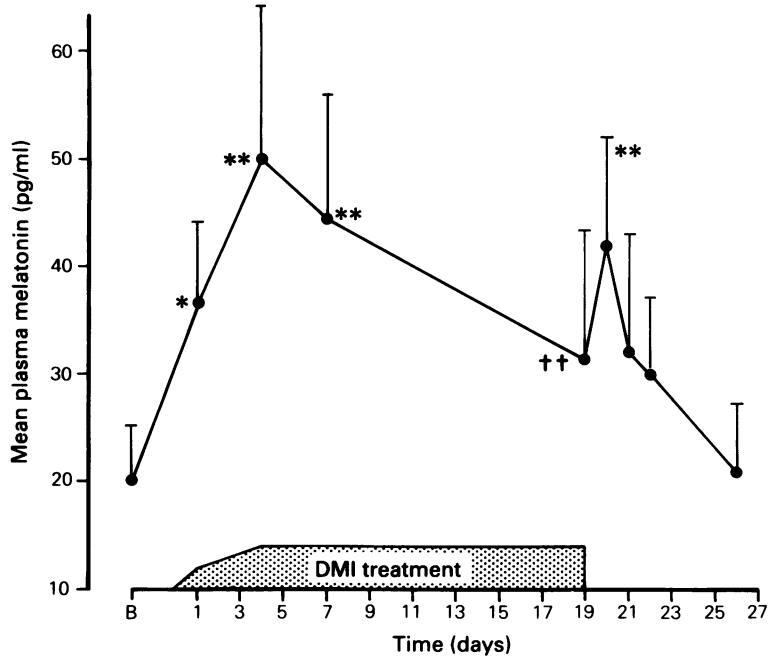

Figure 2 Mean (± s. e. mean) midnight plasma melatonin concentration in 10 normal subjects before (B), during and after 19 days DMI treatment. For treatment schedule see Methods. Midnight melatonin concentration varied significantly during sampling period, $P < 0.005$, (one-way analysis of variance). Significantly greater than B, $^{*}P < 0.025$, $^{**}P < 0.01$.
††Significantly less than day 4, $P < 0.01$, and day 7, $P < 0.025$ (Fisher's LSD test).

during the first week of treatment (Figure 3). Plasma levels on the 19th day of treatment were significantly higher than on day 7 (Figure 3). Following discontinuation of DMI, plasma concentrations fell sharply over the next 24 h (Figure 3). There was no correlation between midnight melatonin and plasma DMI at any period during treatment.

Discussion

The results show that DMI initially increased midnight plasma melatonin levels. While this increase could reflect enhanced pineal NA transmission due to blockade of NA uptake by DMI, we cannot exclude the possibility that DMI might have inhibited the metabolism of melatonin. However, two observations make this unlikely. Firstly, in animal studies where pineal melatonin can be measured directly, TCA treatment, both acute and chronic, produces parallel changes in pineal and plasma melatonin (Wirz-Justice *et al.*, 1980a; Heydorn *et al.*, 1982). Secondly, in the present investigation there was a clear disassociation between plasma DMI levels and plasma melatonin concentration, both during and after treatment. We therefore believe that the changes in plasma melatonin in our study do reflect alterations in pineal noradrenergic transmission (see Introduction).

The increase in plasma melatonin following DMI was not sustained, and by the 19th day of DMI administration the amount of melatonin secreted over 24 hours did not differ significantly from pretreatment values. One interpretation of this finding is that counteractive adaptive changes in pineal noradrenergic synapses have restored NA transmission to normal in spite of the uptake block produced by the DMI.

It is not possible from our study to identify which adaptive mechanism may have restored normal pineal NA function in the presence of DMI. If post-junctional β-adrenoceptor down-regulation were responsible it might be expected that sudden withdrawal of DMI and its uptake blocking effects should lead to a decrease in pineal NA transmission and plasma melatonin levels. However, 24 h after DMI withdrawal melatonin levels were significantly elevated, and then returned to baseline over the next week. This suggests that the removal of DMI caused a 'rebound' increase in NA activity. Such a finding

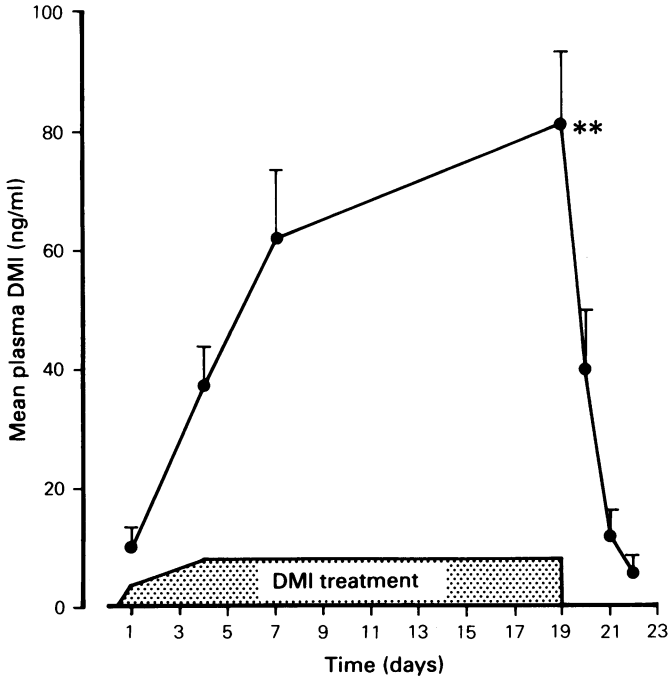

Figure 3 Mean (± s. e. mean) midnight plasma DMI concentration in 10 normal subjects during 19 days DMI treatment. For treatment schedule see Methods. Plasma DMI concentration varied significantly during sampling period, $P < 0.005$, (one-way analysis of variance).
**Significantly greater than day 7, $P < 0.025$, and day 20, $P < 0.01$ (Fisher's LSD test).

is consistent with animal studies demonstrating an increase in central NA impulse flow 24 hours following cessation of chronic TCA treatment (Svensson & Usdin, 1979). In addition, there is evidence in the rat of elevated brain concentration of the NA metabolite 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) 36 hours following DMI withdrawal (Sugrue, 1981). In man, sudden cessation of TCA treatment elevated MHPG concentration in plasma (Charney *et al.*, 1982), but the increase had a slower onset and was more persistent than the elevation in plasma melatonin seen in the present investigation.

From the above studies it appears that a possible explanation for the rebound increase in plasma melatonin is that DMI treatment provokes reduced prejunctional neurone firing (Svensson & Usdin, 1978; McMillen *et al.*, 1980), which in turn leads to the development of subsensitive prejunctional α_2-receptors (Svensson & Usdin, 1978; McMillen *et al.*, 1980). When the DMI is removed there is an increase in prejunctional firing rate, which in the presence of defective α_2-adrenoceptor feedback control leads to a marked increase in NA outflow and plasma melatonin levels. It appears, therefore, that reduced firing of prejunctional NA neurones may be involved in the adaptive changes seen during DMI administration. Interestingly, a previous study has reported that chronic DMI treatment reduces plasma MHPG levels in man (Charney *et al.*, 1981), which would be consistent with this possibility.

The evidence of adaptive change in pineal NA transmission rests on the series of single midnight melatonin samples rather than on 24 h sampling. However, midnight melatonin correlated well with 'total' melatonin secretion where both were measured (see Results). In addition, on the basis of the two 24 h sampling periods, there was no evidence of a shift in the circadian rhythm of melatonin secretion (Wirz-Justice *et al.*, 1980b), which would have complicated interpretation of single midnight values.

Our study fails to provide evidence for a reduction in NA transmission by chronic DMI, instead suggesting that during the first week of treatment at least, NA transmission is increased. While adaptive changes apparently occur at NA synapses during DMI administration their function appears to be to restore homeostasis rather than decrease transmission below normal levels. In contrast a preliminary study in depressed patients has shown that DMI causes a persistent increase in melatonin levels after

3 weeks of treatment (Thompson *et al.*, 1983), suggesting that there may be abnormalities either in NA transmission or in its homeostatic regulation in depressive illness.

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