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Effect of ibuprofen and indomethacin on human plasma melatonin

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Ibuprofen reduced human plasma melatonin (MT) after 2 h when administered orally (400 mg) at 2400h. Increasing plasma concentrations correlated well with increasing inhibition of serum MT levels during this time. Maximum plasma ibuprofen coincided with minimum plasma MT in 3 out of 4 volunteers. Although two volunteers exhibited a partial recovery in MT levels, concentrations after 6 h were significantly less than 0600h values in drug-free volunteers. Administration of ibuprofen (400 mg) at 1800h delayed the nocturnal surge of plasma MT. When a slow release preparation of indomethacin (75 mg) was administered at 1800h, the dark phase rise of plasma MT was completely prevented. Thus the longer acting cyclooxygenase inhibitor exhibited a longer lasting inhibition of plasma MT concentration.

The biosynthesis and secretion of melatonin (MT) by the mammalian pineal gland is initiated by the onset of darkness through noradrenaline action on α- and β-adrenoceptors of the pinealocytes thus stimulating pineal *N*-acetyltransferase activity (NAT) (EC 2.3.1.5) (Klein et al 1983). However, prostaglandins (PGs) may also be involved in this process. Indomethacin, an inhibitor of PG synthesis, greatly impairs the nocturnal increase in rat NAT and MT content (Szabo & Friedhoff 1976; Ritta & Cardinali 1980) and partly inhibits MT release (Cardinali et al 1982). Nanomolar concentrations of PGE_2 in rat pineal cultures increase NAT activity and cyclic (c)AMP accumulation (Ritta & Cardinali 1981). Furthermore, PGs are released in bovine pineal glands by adrenergic stimulation (Cardinali et al 1979) and specific binding sites for PGE_2 and PGF_2 were also observed in these pineals. Thus PGs appear to act at physiological concentrations in pre- and post-synaptic events at the pineal sympathetic neuroeffector junction. In man, MT production appears to be only partly under sympathetic control. The nocturnal increase of MT is prevented by the administration of the β-adrenoceptor blocking drugs, propranolol (Hanssen et al 1980) and atenolol (Cowan et al 1983). Patients with sympatholytic diseases exhibit significantly reduced plasma MT concentrations (Vaughan et al 1979). In addition, Lewy et al (1986) have recently reported reduced human plasma MT after treatment with clonidine, an α-adrenoceptor agonist. However, the human pineal, unlike that of the rat, does not respond to β-adrenergic agonists since no elevation of plasma MT was observed after administration of orciprenaline (Moore et al 1979) or the catecholamine precursor, L-dopa (Wetterberg 1978). So, in man, the mechanism of the sympathetic control of MT synthesis is not fully understood. This study investigates the effect of PG synthesis inhibitors, ibuprofen and indomethacin on human plasma MT concentration to see whether PGs are involved in human MT synthesis.

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Materials and methods

N-[1-Aminoethyl-2-^{3}H]acetyl-5-methoxytryptamine ([^{3}H]MT) 38·6 Ci mmol^{-1} was purchased from New England Nuclear, Dreieich, FRG. *N*-Acetyl-5-methoxytryptamine was obtained from the Sigma Chemical Co., Ltd, Poole, UK. All other reagents were of Analar grade. Ibuprofen (Brufen : Boots) and slow release indomethacin (Indocid-R : Merck, Sharp and Dohme) were both prescribed in standard formulations.

Ibuprofen (400 mg) was administered orally to four fasting healthy male volunteers (age 25–30 years) at 0800, 1200, 1800 and 2400h on four separate days. Blood was collected from cannulae after each drug administration at 0, 30, 40, 50, 60, 75, 90, 105, 120, 180, 240, 300 and 360 min. The blood was centrifuged and stored at −20 °C before assay. In a control experiment, blood was collected from four healthy male volunteers (age 25–37 years) at 0600, 1200, 1800 and 2400h. Plasma MT was measured by RIA using a modification (Bradbury et al 1985) of the method of Ho & Smith (1982). The assay had a sensitivity of 5 pg MT mL^{-1} of plasma and the intra-assay coefficients of variation (CV) were 5·4, 6·5 and 8·0% at 20, 40 and 80 pg mL^{-1}, respectively. The interassay coefficients of variations were 5·7, 8·5 and 8·8% at 10, 40 and 80 pg mL^{-1}, respectively. Recovery of [^{3}H]MT was 75, 85 and 87% at 20, 40 and 80 pg mL^{-1}, respectively. The antiserum did not cross-react with any major indole or with ibuprofen or indomethacin. Plasma ibuprofen was measured by high performance liquid chromatography. An LDC Milton Roy pump was equipped with an ultraviolet detector (Spectromonitor 3. Milton Roy) and a LiChrosorb reverse phase 18/2 (150 × 3·2 mm) 5 μm particle size column (HPLC Technology). The degassed mobile phase, 80% 0·05 M phosphate buffer (pH 8·0) and 20% acetonitrile, was pumped through the column at 1·5 mL min^{-1} (3500–4000 p.s.i.) at ambient temperature. The UV detector was set at wavelength of 225 nm at attenuation of 0·2 AUFS. The plasma was extracted with diethyl ether, separated and the ether phase was evaporated to dryness when the residue was redissolved in 50 μL of the mobile phase. Two major metabolites

(+)-2[4-(2-hydroxy-2-methylpropyl)]phenyl propionic acid and (+)-2[4-(2-carboxypropyl)]phenyl propionic acid, were not extracted by the method and thus were not measured. 20 μL of the plasma extract was injected onto the column. Ibuprofen exhibited a retention time of about 4 min and the internal standard about 6½ min (4-n-pentylphenylacetic acid). The calibration curve was linear over the range 0–50 μg mL^{-1} when ibuprofen standards were passed through the extraction procedure.

The indomethacin slow release preparation (75 mg) was administered to six healthy male volunteers (age 25–30 years) at 1800h. Blood was collected from cannulae at 1800, 1900, 2000, 2100, 2200, 2300 and 2400h, centrifuged and deep frozen before assay. Control blood was collected from four healthy male subjects (age 25–37 years) at 1800, 2000, 2200 and 2400h. Plasma MT was measured by RIA as before.

Results

The zero time plasma MT concentrations at 2400, 0800, 1200 and 1800h immediately before ibuprofen administration exhibited the reported circadian rhythm (Arendt et al 1977; Smith et al 1977; Vaughan et al 1978). The concentration at 2400h was 137 ± 25 pg mL^{-1} (n = 4) which was significantly different from 39 ± 6 pg mL^{-1} at 0800h ($P < 0.005$); from 11 ± 2 pg mL^{-1} at 1200h ($P < 0.002$) and from 29 ± 3 pg mL^{-1} at 1800h ($P < 0.005$). MT concentrations in the drug-free volunteers showed a similar diurnal variation being 113 ± 11 pg mL^{-1} (n = 4) at 2400h; 53 ± 4 pg mL^{-1} at 0600h; 32 ± 11 pg mL^{-1} at 1200h and 23 ± 4 pg mL^{-1} at 1800h. The values of plasma MT at zero time in the ibuprofen-treated volunteers and the values in the drug-free subjects correlated well with those reported earlier (Arendt et al 1977; Smith et al 1977; Vaughan et al 1978).

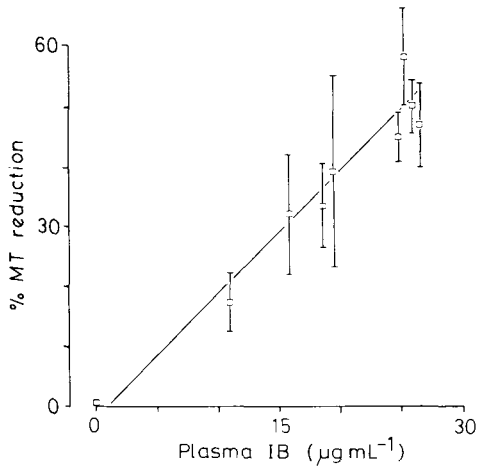

FIG. 1. Variation of percentage reduction of human plasma melatonin (MT) level with plasma concentration of ibuprofen (IB) during 2 h after administration. Each point represents the mean of 4 individuals ± s.e.m. Regression line of y on x (r = 0·976).

The effects of ibuprofen given at 2400h significantly reduced the zero time plasma MT levels by 51 ± 7% in 120 min (n = 4) ($P = 0.03$). Fig. 1 illustrates during this time a greater inhibition of plasma MT concentration with increased plasma ibuprofen levels (regression line of y on x : r = 0·976). Two volunteers (Fig. 2A, B)

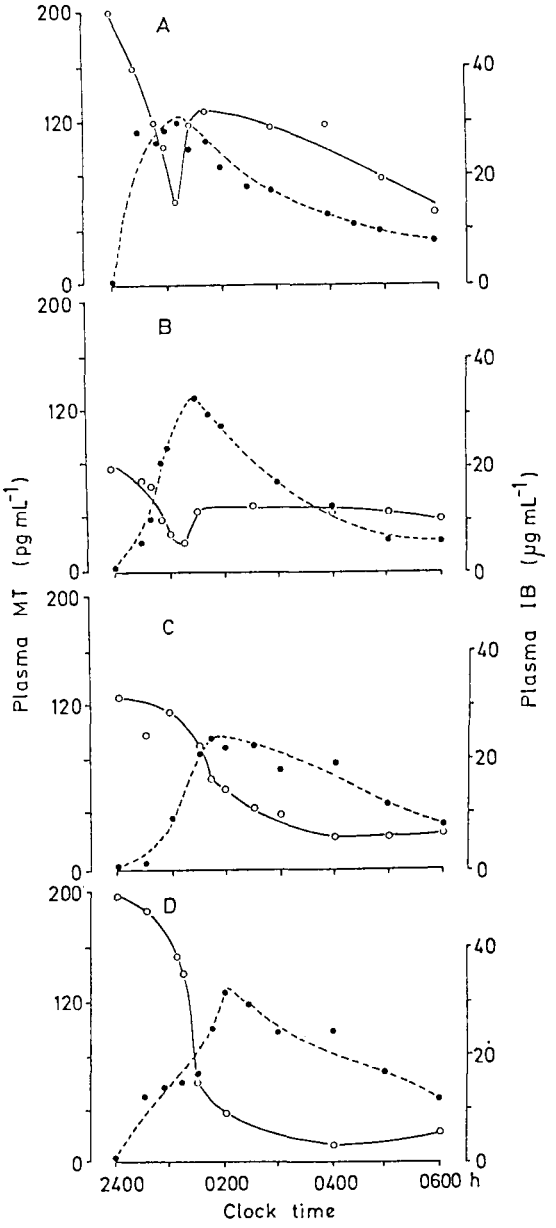

FIG. 2. Curves A–D represent the variation in human plasma melatonin (MT) (○——○) and ibuprofen (IB) concentrations (●– – –●) with time from the time of administration of the drug at 2400h until 0600h in four volunteers. Each point represents the mean of two determinations.

exhibited at 75 min a 70 and 73% decrease in plasma MT when compared with their 2400h MT concentrations. These minima coincided with peak ibuprofen concentrations (31 and 39 μg mL^{-1}, respectively). After 90–120 min plasma MT concentrations partially recovered exhibiting only a 35 and 38% decrease, respectively. After 6 h, plasma MT had gradually declined to values which represented a 70 and 50% inhibition of 2400h levels. In the two other volunteers (Fig. 2C, D), MT concentrations after 120 min exhibited 52 and 82% inhibition on their 2400h values at peak ibuprofen concentrations of 25 and 32 μg mL^{-1}, respectively, although only the latter peak coincided approximately with minimum MT levels. However, there was no partial recovery in either case and plasma MT concentration remained low until 0600h. The average MT level at 0600h in the drug-treated group was 34 ± 4 pg mL^{-1} (n = 4) which was significantly lower than the MT concentration in the drug-free control volunteers of 53 ± 4 pg mL^{-1} ($P < 0.05$).

Similar but smaller effects on plasma MT concentrations were observed when the ibuprofen was administered at 1800h. The evening rise in plasma MT was delayed between 2000–2200h. The 2400h levels (6 h after dosage) were 68 ± 14 pg mL^{-1} (n = 4) and were lower than 137 ± 25 pg mL^{-1} (n = 4) recorded in the same volunteers a few days earlier at 2400h before drug administration. However, the average decrease did not quite reach significance ($P = 0.07$). The administration of ibuprofen at 0800 and 1200h had no observable effect on plasma MT concentration probably because normal values at these times are near the sensitivity of the RIA for MT.

The effect of the slow release preparation of indomethacin (75 mg) administered at 1800h was to inhibit completely the nocturnal rise of dark phase plasma MT when compared with controls. Fig. 3 indicates that plasma MT concentration in the drug-treated volunteers (n = 6) remained low between 6 ± 1 and 12 ± 2 pg mL^{-1} from 1800 to 2400h, respectively. The drug-free control group (n = 4) exhibited the normal nocturnal rise being 23 ± 14, 30 ± 4, 57 ± 8 and 113 ± 29 pg mL^{-1} at 1800, 2000, 2200 and 2400h, respectively. The difference at 2200 and 2400h between indomethacin-treated and drug-free controls was highly significant ($P < 0.001$).

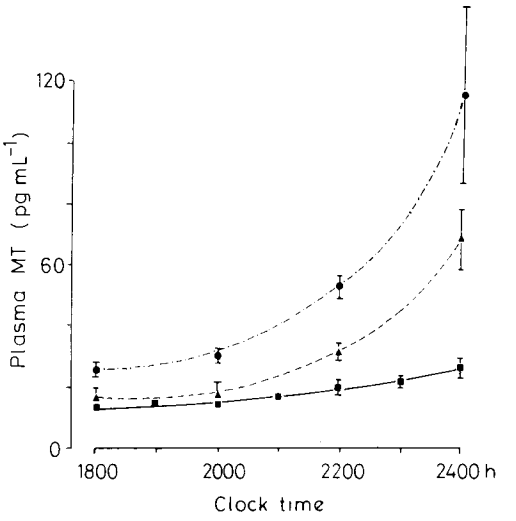

FIG. 3. Variation of human plasma melatonin (MT) with time from 1800–2400h of control volunteers (n = 4) (●–·–·–●), slow release indomethacin (n = 6) (■——■)- and ibuprofen (n = 4) (▲– – – –▲)-treated subjects at 1800h. Each point represents the mean ± s.e.m.

Discussion

In untreated subjects, human plasma MT concentrations at 2400h are still undergoing the nocturnal increase reaching a maximum at about 0200h (Arendt et al 1977; Smith et al 1977; Vaughan et al 1978; Wetterberg et al 1978). After peaking, MT concentrations only begin to decline at the end of the dark phase at 0500–0600h. The effect of ibuprofen was to reduce significantly the 2400h MT concentrations at 0200h, a time when they are usually peaking. This is consistent with the argument that ibuprofen inhibits prostaglandin synthesis by inhibiting the cyclooxygenase enzyme system and the consequent lack of prostaglandin in the pineal gland results in a reduction in the stimulation of MT synthesis. However, the possibility exists that ibuprofen may exert its action at some other site, possibly in the neuronal pathways from the suprachiasmatic nuclei in the hypothalamus to the pineal gland. Indeed, many pharmacological agents interfere with the passage of neural impulses in the above multisynaptic pathway (Zatz 1981).

However, two of the four volunteers exhibited a partial recovery in MT concentration, the maximum inhibition coinciding with peak drug concentration. This transient effect may be associated with the short time to peak plasma ibuprofen concentration of 40 and 75 min in these two subjects. In the other two volunteers, plasma MT was suppressed over a longer period with no partial recovery and was associated with longer time to peak ibuprofen concentrations of 105 and 135 min.

However, administration of a slow release preparation of indomethacin (another cyclooxygenase inhibitor) at 1800h, gave a complete blockade of the nocturnal increase of human plasma MT. A similar slow release preparation of indomethacin when administered at 2000h to rheumatoid diseased patients exhibited a time to peak indomethacin concentration of 4·9 h (Guissou et al 1983). Thus the long acting indomethacin has a longer inhibitory effect on dark phase MT concentration than the shorter acting ibuprofen.

This reduction of dark phase human plasma MT is similar to that seen in rats (Ritta & Cardinali 1980) when indomethacin reduced nocturnal plasma MT by 30%. Since indomethacin also inhibited dark phase pineal NAT (Szabo & Friedhoff 1976) and hydroxy-

indole-*O*-methyl transferase activity (Ritta & Cardinali 1980), and since noradrenaline released PGs from bovine pineal explants (Cardinali et al 1979), it was suggested by Cardinali et al (1982) that the PGs may be involved in the pre- and/or postsynaptic events in the pineal gland, leading to MT synthesis. It was also suggested that the structural similarities between MT and indomethacin may interfere with pineal binding of MT. However, ibuprofen does not structurally resemble MT and therefore the latter suggestion is not a probable explanation. The fact that, in man, both ibuprofen and indomethacin administration produce a reduction in plasma MT concentration supports the theory that the PGs may be involved in the biochemical events leading to human MT synthesis.

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Metabolic *N*-oxidation of metronidazole

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Metronidazole when treated at the *N*-3 nitrogen with a mixture of hydrogen peroxide and acetic acid, or liver homogenate preparations, yields the *N*-3 oxide as identified by thin-layer chromatographic analysis on silica gel G, R_F 0·62 in ethanol–chloroform–ammonia (50 : 49 : 1), by chemical reduction with sulphur dioxide, and by ultra-violet spectrophotometry and nuclear magnetic resonance spectroscopy. Incubation of metronidazole at 37 °C with rat liver 10 000*g* supernatant fortified with cofactors gave a product with identical chromatographic and UV spectral data suggesting that metronidazole like other tertiary amine drugs undergoes microsomal *N*-oxidation.

The exact mechanism of the anti-protozoal action of metronidazole is not clear. Apparently the drug's biological activities are related to the reduction of its nitro group. Mutagenic activity towards a variety of bacteria has been reported by Rosenkranz & Speck (1975) who also showed that the mutagenicity was detectable in a strain of the Ames histidine auxotrophs of *S. typhimurium* that lacked nitroreductase activity. Similarly a rare strain of *Bacteroide fragilis,* relatively resistant to metronidazole, was found to have greatly diminished nitroreductase activity (Tally et al 1979).

The nature of the metabolites remains a matter of controversy. Either of the two side chains can be oxidized in the liver to give methyl alcohol or 'acid' metabolites (Connor et al 1977), but only the alcohol metabolite was detected in the serum of a subject with normal renal function (Wheela et al 1978). The alcohol metabolite has also been implicated as a potent mutagen (Connor et al 1977).

Some tertiary amines have been shown to be metabolized via *N*-oxidation (Beckett et al 1971; Dagne & Castagnoli 1972). Metronidazole, with two tertiary nitrogen centres, has the possibility of *N*-oxidation by microsomal enzymes so this has been investigated.

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