Psychopharmacology (1986) 88:493–495

Psychopharmacology

Circadian rhythm of serotonin transport in human platelets

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Abstract. The circadian rhythm of serotonin active transport in human platelets was investigated in ten healthy men, aged 27–35 years. Blood was collected at 08.00, 14.00, 20.00, 02.00 and 08.00 hours the next morning. Simultaneous evaluation of the mean platelet volume, platelet distribution width, platelet distribution skewness and platelet number in whole blood was performed. K_m and V_{max} for serotonin transport varied considerably among individuals over 24 h. However, the mean values and distribution of these kinetic parameters were reduced at 02.00 hours. All platelet size or number parameters were stable and normal over 24 h; therefore, the reduction in mean K_m and V_{max} values at 02.00 h is not related to morphological platelet differences but either to platelet intrinsic factors or plasmatic variables. Knowledge of the affinity and capacity of serotonin transport throughout the diurnal cycle is important for future comparisons with depressed patients as well as other hormonal rhythms in patients and healthy humans.

Key words: Circadian rhythm – Serotonin – Transport – Platelets

Depressive illness has been related to biological rhythm disturbances, either in shape or in phase (Carruthers et al. 1976; Wher et al. 1979, 1980, 1982). Inherent in this illness are diurnal changes in affect, sleep, activity, appetite, bowel movements, etc. The phase advance theory in depression is based on a shift that can be observed in steroids (Beck-Friis et al. 1981; Wetterberg et al. 1981), 3-methoxy-4-hydroxyphenyl glycol (Wher et al. 1980) and melatonin (Wetterberg et al. 1984) cycles, besides physiological parameters such as temperature (Wher et al. 1980) and sleep cycles (Wher et al. 1979).

Serotonin uptake by platelets of depressive patients has repeatedly been found to be decreased (Coppen et al. 1978; Malmgren et al. 1981; Meltzer et al. 1981; Modai et al. 1984; Ross et al. 1980; Scott et al. 1979; Tuomisto et al. 1976; Tuomisto et al. 1979). Many investigators suggested that it reflects a decreased number of binding sites, represented by the decreased V_{max} rather than change in affinity as represented by a similar K_m value in the populations compared (Meltzer et al. 1981; Modai et al. 1984; Tuomisto et al. 1976; Tuomisto et al. 1979). In none of these studies was any consideration given to the possible circadian rhythm of the serotonin uptake, especially when depressive patients have many rhythm disturbances, as mentioned above. If a pronounced rhythm could be established in normal populations, similar investigations in depression could give answers to the question whether the reduced serotonin transport by platelets from depressives reflect a decreased number of binding sites or a shift in the circadian rhythm.

This work was carried out in order to establish the rhythm and variability of serotonin uptake kinetics in healthy individuals during day and night.

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Subjects and methods

The experiment was carried out in Sweden during the months of August to November. Ten healthy men, aged 27–35 years, participated in the study. A thorough physical examination, meticulous medical history and routine laboratory blood tests were carried out prior to blood sampling. Only healthy, drug-free individuals participated in the study.

All subjects continued their usual everyday activities but refrained from hard physical effort or changes in their dietary habits. During the night they slept at the Psychiatric Ward, Karolinska Hospital.

Materials. ACD solution: citric acid 1.37%, sodium citrate 2.5%, anhydrous glucose 2%, pH 4.5.

5-Hydroxytryptamine 31-^{14}C-creatinine sulphate (^{3}H)-5-HT) (specific activity 55 mCi/mmol): Amersham International, Amersham, UK. The ethanol added by the manufacturer was removed by freeze drying. Picofluor®: Packard Instrument Co., Downers Grove, Illinois, USA.

Experimental procedures. Blood was collected by a 1.4 mm stainless steel cannula at 08.00, 14.00, 20.00, 02.00 and 08.00 hours the following morning, and immediately assayed.

Blood (9 ml) was sampled in plastic tubes containing 1 ml ACD solution. Platelet-rich plasma was prepared by centrifugation of the blood at 190 *g* for 15 min at room temperature. PRP (300 µl) was temperature-equillibrated for 5 min at 37° C and then incubated with (^{14}C)-5-HT (25 µl) for 60 s. The final concentrations of (^{14}C)-5-HT were 0.75, 0.88, 1.15 and 2.93 µM in six experiments and 0.5, 0.86, 1.15 and 1.83 µM in the remaining four experiments. Each concentration was assayed in duplicate sam-

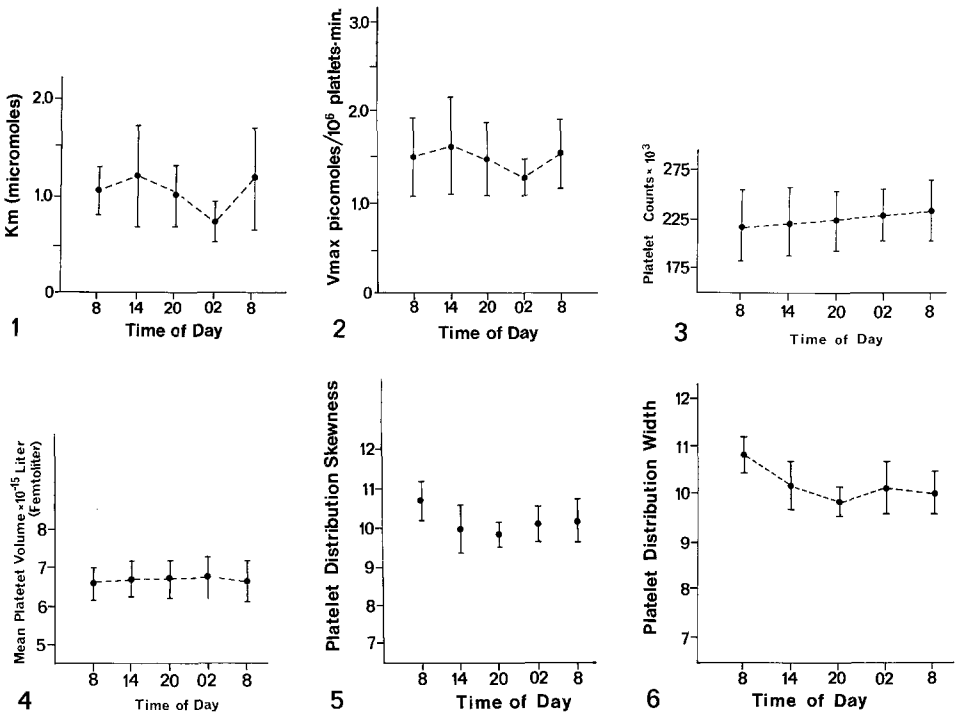

Fig. 1. K_m values during 24 h in ten healthy men aged 27–35 years

Fig. 2. V_{max} values during 24 h in ten healthy men aged 27–35 years

Fig. 3. Platelet counts in whole blood during 24 h in ten healthy men aged 27–35 years

Fig. 4. Mean platelet volume during 24 h in ten healthy men aged 27–35 years

Fig. 5. Platelet distribution skewness during 24 h in ten healthy men aged 27–35 years. Normal range is between 8.8 and 11.2

Fig. 6. Platelet distribution width during 24 h in ten healthy men aged 27–35 years. Normal range is between 8.8 and 11.2

ples. Additional duplicates of all concentrations of PRP, preincubated with imipramine (10^{-5} M), were used in order to evaluate the magnitude of unsaturable transport (Malmgren 1984).

The uptake was terminated by adding 0.1 ml 4.5% formaldehyde-EDTA solution to the samples, which immediately afterwards were cooled on ice. The test tubes were then filled with 1.5 ml 0.9% saline solution and the mixtures were filtered through GF/C filters (Whatman Ltd., Springfield Mill, Madistone, UK) under continuous water suction. The filters and the test tubes were rinsed twice with 2.5 ml saline solution. The filters were transferred to scintillation vials to which 4 ml Picofluor was added. The (^{14}C)-activity was determined in a liquid scintillation spectrometer (Tricarb 2425, Packard Instrument Co.).

All uptake values are given after subtraction of the corresponding blank values. The kinetic parameters K_m and V_{max} were determined according to the method described by Eadie and Hofstee.

The platelet counts were obtained using an automatic cell counter (Linson 431 A, combined with a Linson 433 Monitor, LIC, Stockholm, Sweden). The pH of the PRP was determined with the aid of a pHM 82 pH-meter fitted with a GK 2321 glass electrode (Radiometer, Copenhagen, Denmark).

The platelet size distribution was determined with a Contraves Cell Analyzer 8016 (Contraves Ltd., Zurich, Switzerland). The instrument performs electronic counting in 2×125 channels. The discriminator level is automatically set to minimize background scattering.

Results

The mean values of K_m and V_{max} for serotonin transport are illustrated in Figs. 1 and 2. Platelet counts in whole blood and their size distribution parametes are illustrated in Figs. 3–6.

Figure 1 illustrates a reduction in K_m at 02.00 h below 0.9 μM, while day values were between 1.1 and 1.2 μM. Figure 2 illustrates a reduction in V_{max} at 02.00 h below 1.3 pmol/10^6 platelets per min, while day values were between 1.5 and 1.6 pmol/10^6 platelets per min.

Although the reductions in mean K_m and V_{max} at 02.00 h were mild (25–33% for K_m and 15–25% for V_{max}), all ten individuals had their lowest K_m and V_{max} values at this time of night. However, distribution (C.V. of $V_{max} = 16\%$, $K_m = 22.5\%$ compared to 24–35% and 24–43%, respectively) was significantly ($P < 0.001$) reduced in both K_m and V_{max} at this time of night. The number of platelets in whole blood (Fig. 3) was very stable, as was the mean platelet volume (around 6.7 FL) (Fig. 4), while the platelet distribution skewness (Fig. 5) and width (Fig. 6) deviated slightly and insignificantly (up to 8%), and remained in the normal range.

Each individual had a unique K_m and V_{max} circadian rhythm but, as mentioned above, all showed the lowest point at 0.200 h.

Discussion

The K_m value reflects the affinity of serotonin for its uptake carriers and V_{max} the transport capacity of these carriers. Both of these parameters varied considerably during the diurnal cycle. This could be attributed to intrinsic platelet changes or plasmatic factors. It is not related to size parameters or number of platelets, as they were stable and normal over 24 h. The mild reduction in platelet distribution skewness and width at 20.00 h does not correlate with the reduction in K_m and V_{max} values at 02.00 h.

Laboratory techniques, which were carefully controlled, only account for 5% deviation in results. Mean and individual values of K_m and V_{max} decreased at 02.00 h. At the same time, distribution of both values was significantly reduced both in mean and individual values. These changes are concomitant with changes in melatonin secretion (Wetterberg et al. 1984). Melatonin peaks significantly at around 02.00 h and was found to be flattened in depressed patients (Wetterberg et al. 1984). Melatonin and serotonin are biochemically related, and the occurrence of an interdependent mechanism in the regulation of these hormones could be conceivable. Perhaps a reduction of serotonin presynaptic re-uptake in the brain serves the goal of increasing melatonin secretion from pineal body. The melatonin synthesis is regulated by light quantities through the polyneuronal pathway from the retina via the suprachiasmatic nuclei to the pineal body (Klein and Weller 1972).

The reduction in V_{max} and K_m of serotonin transport during the night may also be related to reduction in light, likewise melatonin secretion.

Another major variable besides light quantity in this population was alertness and activity during daytime versus sleep and rest at night. As transport of serotonin is an active process and could be influenced by other hormonal levels in plasma, such as endogenic serotonin, cathecholamines, steroids, etc., it is of great interest to investigate whether there are any concomitant changes in hormonal activity and uptake in the same population. Any time- or phase-related changes in these circadian rhythms would suggest a possible relation between serotonin uptake and these hormonal activities. In addition, such a comparison between healthy people and depressed patients would add more evidence for a possible connection between hormonal changes and serotonin uptake in depression. For example, a shift in the observed decrease at 02.00 h in serotonin uptake by platelets of healthy men to 08.00 h in depressive patients would explain the decrease in uptake observed by many investigators who have been collecting blood in the morning hours (Coppen et al. 1978; Malmgren et al. 1981; Meltzer et al. 1981; Modai et al. 1984; Ross et al. 1980; Tuomisto et al. 1979).

As variability between individuals is lowest at 02.00 h both for K_m and V_{max} values, it seems that sampling at this time of night is more reliable when populations are compared. The explanation for this phenomenon is not known, as all the possible plasmatic and intrinsic factors which influence this process are obscured.

Acknowledgement. Acknowledgement is made to Yuval Janai for skillful biochemical assistance.

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Received May 30, 1985; Final version October 6, 1985