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Diurnal Concordance of Human Platelet Serotonin Content and Plasma Alpha-1-Acid Glycoprotein Concentration

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MEYERSON, L. R., R. STRANO AND D. OCHERET. *Diurnal concordance of human platelet serotonin content and plasma alpha-1-acid glycoprotein concentration.* PHARMACOL BIOCHEM BEHAV **32**(4) 1043–1047, 1989.—The diurnal rhythm of platelet serotonin (5-HT) content and plasma alpha-1-acid glycoprotein (AGP) was investigated in twelve healthy male volunteers aged 19–30 years. Platelet 5-HT content and plasma AGP concentration varied across a twenty-four hour sampling period in a manner consistent with a circadian pattern. Platelet 5-HT levels rose from baseline at 5.00 hr to a peak at 14.00 hr and slowly declined to baseline during the night-time hours. A concordant pattern was observed for plasma AGP with peak concentrations occurring between 8.00 hr and 14.00 hr. These findings support the notion that AGP is a positive endogenous allosteric stimulator of platelet 5-HT transport, since there is a direct relationship between platelet 5-HT levels and plasma AGP concentration. A theoretical model is presented to explain the diurnal effects of AGP on the platelet 5-HT transporter.

Serotonin Platelets Alpha-1-acid glycoprotein Circadian Endogenous modulator

CIRCADIAN rhythmic variations of serotonin (5-HT) uptake (18,34) and levels (6,25) have been reported. Alterations in such rhythms have been the focus of numerous clinical biological marker studies. For example, 5-HT uptake by platelets derived from depressed patients has repeatedly been found to be decreased (13, 16, 26, 29, 31, 32) and a disturbance of its rhythm in depressives compared to controls has also been observed (9,22).

The site labeled by [^{3}H]-imipramine in human platelets has been suggested to be allosterically coupled to the 5-HT transporter (17,27). Other views have been advanced suggesting that 5-HT uptake inhibitors block the substrate recognition site for the 5-HT transporter and additionally bind to sites close to the substrate binding site (14).

It has been demonstrated in a number of laboratories that a reduction in the B_{max} of platelet [^{3}H]-imipramine binding sites occurs in depressed patient populations [(12) for review]. In contrast, studies have also shown an increase in [^{3}H]-imipramine binding (15) while others have observed no significant differences between depressed patients and controls (5, 9, 33). A circadian rhythm has been described for this binding site in human platelets (19) which is opposite to the rhythm observed in rabbit platelets (8).

Evolving studies of platelet and brain [^{3}H]-imipramine binding have spawned research for possible endogenous factors which can modulate 5-HT uptake (2–4, 24, 28, 30). Recently, we have purified a glycoprotein from human plasma which not only competes with imipramine for this allosteric modulatory site but enhances 5-HT uptake velocity (1). Based on a compendium of analytical data we have identified this protein as alpha-1-acid glycoprotein (AGP) (1). Most recently, we have observed a 25% increase in AGP in plasma from drug-free patients with major depression (DSM-III) compared to normal controls (20).

An earlier study in normal volunteers evaluated the possible diurnal variation of AGP. Some subjects showed systematic deviations in serum AGP concentrations over time (37). With the known ability of AGP to stimulate platelet 5-HT transport, the present study was conducted to determine if there is a concordance between the diurnal rhythm in platelet 5-HT content and plasma AGP concentration.

METHOD

Reagents

Serotonin hydrochloride (5-HT) was obtained from Research Biochemicals Inc.; human alpha-1-acid glycoprotein (AGP) from Sigma Chemical Company; N-methyl serotonin from Regis Biochemicals, Inc. All other chemicals were of the highest purity commercially available.

Subjects and Protocol

The study was carried out on the Ramapo College of New Jersey campus during the months of September and October.

TABLE 1

STANDARDIZED DIET USED IN PROTOCOL

Meal	Time Served	Carbohydrates (g)	Protein (g)	Fat (g)
Breakfast	8.00 hr	45.5	18.5	23.4
Lunch	12.00 hr	46.7	25.0	21.1
Dinner	18.00 hr	58.2	26.9	13.8
Snack	22.00 hr	45.9	18.3	37.3

Thirteen male college students, aged 19–31 years, volunteered for the study. Informed and written consent was obtained from all subjects. The attending physician examined the subjects prior to the study and all were found to be in excellent health. Only nonsmoking and drug-free individuals with no history of mental illness participated. All subjects were instructed to go about their usual daily activities for the week prior to the study. The night before the study the subjects were asked to refrain from ingesting food and liquids from 11:30 p.m. until the start of a controlled diet the following morning. The subjects arrived at the campus health service center for blood collection at 8.00 hr, and a breakfast was served at the college food service dining area. The subjects reported back to the health center for blood drawings at 11.00 hr, 14.00 hr, 17.00 hr, 20.00 hr and 23.00 hr. Controlled diets (breakfast, lunch, dinner and snack) were prepared by the college dietitian. The above stated diet of each participant was approximately 1850 calories. The precise breakdown of food classes in grams is given in Table 1. Group sleeping accommodations for the subjects were made by the college residency office. Scheduled bed-time was at 23.00 hr. The subjects were briefly awakened under dim illumination at 2.00 hr and 5.00 hr for blood collection.

Laboratory Methods

Blood was drawn at three-hour intervals by venipuncture into sterile 3-ml Vacutainer tubes containing 7.5% EDTA. A Latin square design was used to determine the order in which the subjects had their blood drawn. Individual samples of whole blood were centrifuged at $300 \times g$ for 15 min within one hour after venipuncture. An aliquot of the supernatant (platelet-rich plasma) was taken for platelet counting using a Coulter Counter Model ZF (Coulter Electronics, Hialeah, FL). The platelet-rich plasma was further centrifuged at $5{,}000 \times g$ for 15 min to yield a platelet pellet and platelet-free plasma supernatant. The resultant platelet pellet was dispersed using a Tekmar ultra turrax tissuemizer in 3.0 ml of distilled water. The platelet preparation was subjected to thirty, 1-sec ultrasound bursts (Branson sonifier) and stored frozen at -20°C along with the platelet-free plasma samples.

Alpha-1-Acid Glycoprotein (AGP) Analysis

Platelet-free plasma samples were thawed and assayed for AGP concentration using a commercially available radial-immunodiffusion technique (Nor-Partigen Kit, Calbiochem-Behring).

Platelet 5-HT Analysis

Platelet 5-HT concentration was assessed employing the following procedure. Four hundred and eighty μl of platelet preparation was extracted with 125 μl sulfosalicylic acid (100 g/l) and centrifuged at $8500 \times g$ for 5 min. The supernatant was filtered through a 0.45 micron Millex filter and subjected to HPLC analysis as described below.

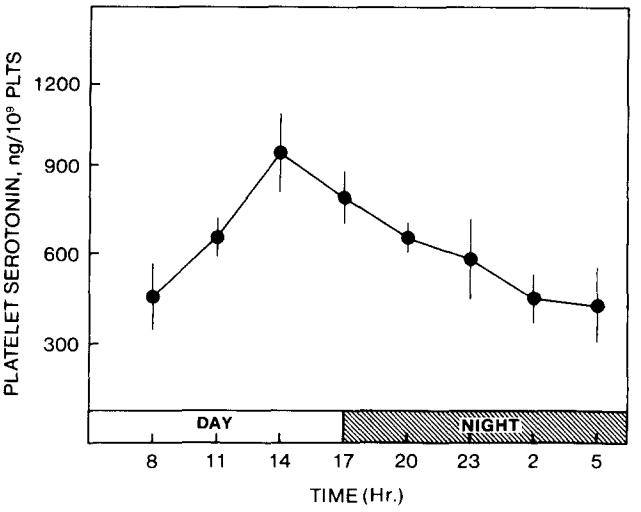

FIG. 1. Diurnal variation of platelet serotonin concentration in normal human males. Data points represent mean values ± S.D. for twelve individuals at eight different time points.

HPLC Instrumentation

Chromatography and estimation of 5-HT concentration was performed using a Waters Associates (Milford, MA) modular liquid chromatograph equipped with a refrigerated (4°C) Model 712 WISP automatic injector, Model 6000A solvent delivery system, Model 740 data module and a Model LC-4B amperometric detector (Bioanalytical Systems, West Lafayette, IN). All separations were performed on an Altex Ultrasphere I.P. 15 cm C-18 reverse phase column equipped with an inline filter (2 micron frit). Elution of serotonin and standards was achieved with an isocratic buffer system consisting of 0.1 M sodium phosphate buffer pH 4.25 containing 200 μM EDTA and 4% methanol. Typically, 200 μl volumes of extracted platelet preparation was injected onto the column and eluted at a flow rate of 2.0 ml/min and a detector sensitivity of 10 nA/V at +0.70 applied voltage. N-methyl serotonin (500 ng in 10 μl) was used as the internal standard.

Statistics

Statistical tests, the Kolmogorov-Smirnov test of normality, analysis of variance (ANOVA), analysis of covariance (ANCOVA), linear regression, and Pearson's correlation were performed using RS1, a statistical analysis program (BBN Software Corp., Cambridge, MA).

RESULTS

Thirteen volunteers entered the study and twelve completed the protocol. One of the volunteers was withdrawn from the study due to an acute respiratory infection.

Clear variations in platelet 5-HT concentrations were observed over a 24-hour period (Fig. 1). The 5-HT platelet concentration rose from 8.00 hr, peaked at 14.00 hr, and slowly tapered to basal levels (2.00–5.00 hr). Baseline (5.00 hr) platelet 5-HT concentration was 432 ± 63 ng/10^9 platelets (mean ± S.D., $n = 12$) while the peak (14.00 hr) was 944 ± 109 ng/10^9 platelets (mean ± S.D.,

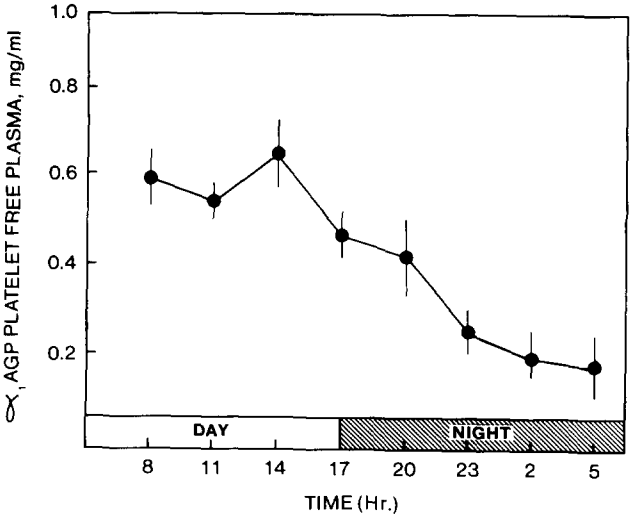

FIG. 2. Diurnal variation of platelet free plasma alpha-1-acid glycoprotein in normal human males. Data points represent mean values ±S.D. for twelve individuals at eight different time points.

n = 12). Our platelet 5-HT values are consistent with that reported by Stahl *et al.* (29) who found 697 ± 34 ng 5-HT/10^9 platelets (normal controls) when sampled between 8.00 hr and 10.00 hr.

A similar variation in plasma AGP concentration was observed (Fig. 2). The plasma AGP concentration peaked between 8.00 hr and 14.00 hr and slowly tapered to baseline levels during the evening. Baseline (5.00 hr) AGP plasma concentration was 0.17 ± 0.06 mg/ml (mean ± S.D., n = 12) while the peak (14.00 hr) was 0.66 ± 0.06 mg/ml (mean ± S.D., n = 12).

The distribution of platelet 5-HT and plasma AGP values did not differ significantly from normal (Kolmogorov's D = 0.084, $p = 0.23$; D = 0.105, $p = 0.48$, respectively). For purposes of comparison and analysis, data were expressed as percentages of the 14.00 hr measurement on the same subject and means ± S.D. at each time point are shown in Fig. 3. Following arcsin transformation, the distributions of both platelet 5-HT and plasma AGP percent of control values did not differ significantly from normal (Kolmogorov's D = 0.134, $p = 0.09$; D = 0.108, $p = 0.27$, respectively). One-way parametric ANOVA found highly significant differences among the means of platelet 5-HT levels (F = 24.67, $p < 0.001$), and among the means of plasma AGP levels (F = 63.37, $p < 0.001$) taken at the various times.

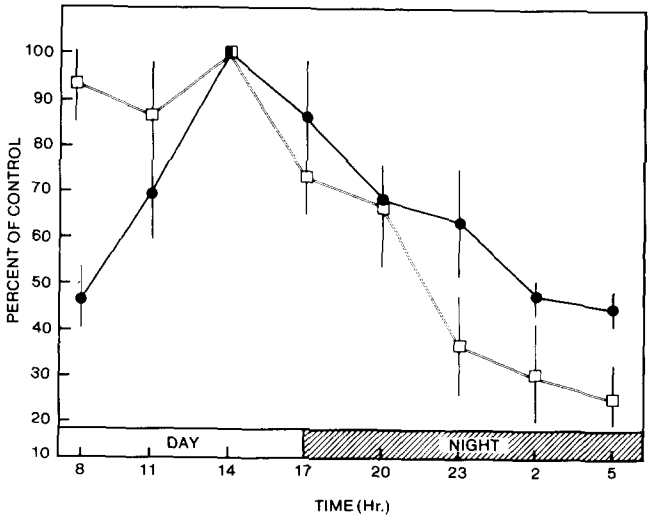

FIG. 3. Mean percent change from 14.00 hr peak of platelet 5-HT and plasma AGP across a 24-hr period in normal human males. Data points represent the mean percent change ±S.D. from 14.00 hr peak for twelve individuals. (●) = mean % change platelet 5-HT; (□) = mean % change plasma AGP.

Linear regression analysis performed on the means of transformed % of control platelet 5-HT and plasma AGP levels showed a correlation coefficient of 0.64 (F = 4.32, $p = 0.09$). ANCOVA was used to analyze the relationship between time-concentration curves for platelet 5-HT levels and plasma AGP levels, and detected no significant difference between the slopes of the two curves (F = 0.20, $p = 0.33$).

ANCOVA of the descending limbs (14.00–5.00 hr) of the 5-HT and AGP time-concentration curves showed no significant difference between their slopes (F = 0.001, $p = 0.98$). Linear regression analysis of the descending limbs of both concentration curves showed that concentrations of platelet 5-HT and plasma AGP were highly significant predictors for one another (r = .97, F = 58.4, $p = 0.002$), during this period. Thus, with the exception of 8.00 and 11.00 hr values, platelet 5-HT levels and plasma AGP concentrations were highly correlated.

A theoretical model of circadian rhythmicity of platelet 5-HT transport is proposed in Fig. 4. During the day, plasma AGP levels are highest and this natural modulator acts as an agonist to allosterically stimulate the platelets to recapture more circulating 5-HT. During the evening hours AGP levels are reduced to a point where the transporter operates at a lower velocity which would result in lower platelet concentrations of 5-HT. In the event the allosteric antidepressant antagonist imipramine (16) is present, the transporter velocity would be further reduced resulting in an even lower platelet 5-HT concentration in the dense core granules.

DISCUSSION

The human circadian system is a control mechanism in which biochemical, physiological and biobehavioral changes occur and reoccur in a precise temporal framework. This system could provide the infrastructure for mechanisms involved in both normal physiology as well as pathophysiology.

In mammalian species, the majority of 5-HT resides outside the central nervous system in the enterochromaffin cells, platelets, liver, spleen, thyroid (7) and participates in physiological events in the cardiovascular and gastrointestinal systems.

AGP is present in normal human plasma at a concentration of 0.1–1.2 mg/ml which is equivalent to 2–20 μM. In vitro, AGP stimulates the reuptake of 5-HT in washed platelets with an EC_{50} of approximately 7 μM (1). Thus, it appears that varying the concentration of circulating plasma AGP is within the range to have physiological consequence (i.e., accelerate or decelerate) on platelet 5-HT reuptake. The data presented in this report suggest there is a time-dependent parallelism between platelet 5-HT level and plasma AGP concentration. During the light hours of the day both platelet 5-HT and plasma AGP reach a peak and slowly taper during the dark night cycle. If increased plasma concentration of AGP does stimulate platelets to recapture greater amounts of 5-HT, then the predicted result would be increased platelet 5-HT. The observed results support a participative role of plasma AGP in regulating platelet 5-HT levels.

Peripherally, AGP is produced by the liver and is subject to induction (11). Recently, we have shown that mRNA levels coding for AGP are three times greater in the spontaneously

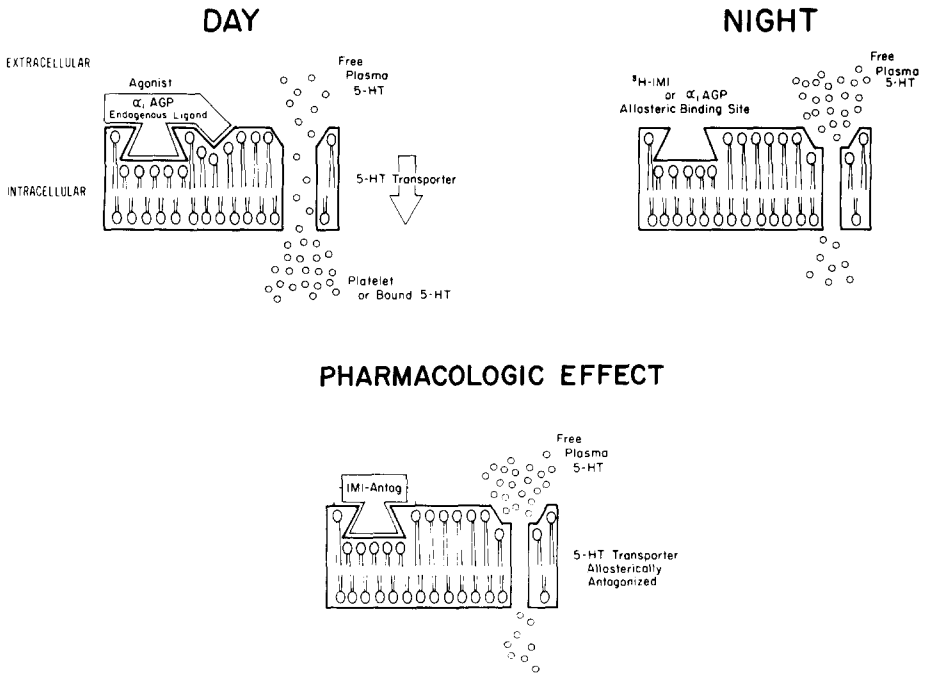

FIG. 4. The allosterically modulated 5-HT transporter: Effects of AGP and imipramine. Theoretical model of the platelet 5-HT transporter and attendant allosteric modulator site. Model depicts accelerated transporter state during the day and normal night state and decelerated state during pharmacological intervention with imipramine.

hypertensive rat (SHR) than in normotensive control rats (KWy) (21). The genetic hypertension in SHR's may be related to abnormal metabolism (i.e., reuptake or biotransformation) of 5-HT in these animals resulting in increased vasomotor tone.

Earlier studies (21) demonstrated that in the rat hypothalamus, frontal cortex and brain stem, peak 5-HT concentrations occurred during the daily period of light. Moreover, Wirz-Justice and co-workers (36) found a diurnal variation in human platelet 5-HT with a minimum in the afternoon and higher values in the morning and evening. This study, however, did not evaluate the time frame between 19.00 hr and 8.00 hr. Wirz-Justice and colleagues also found a circadian rhythm in [^{3}H]-imipramine binding in rat hypothalamus (35). Imipramine binding was highest in the dark phase, an observation which is consistent with our findings of lower night-time AGP levels. Taken together, these findings concur with our observation of a parallelism between platelet 5-HT content and plasma AGP concentration.

Although the exact mechanism regulating 5-HT levels in brain is not known, it is possible that the presynaptic 5-HT transport system may be activated also by AGP. Recently, we have shown the presence of an AGP-like mRNA species in rat brain (21). For AGP to play a similar role in the CNS as in the periphery, it would be expected that AGP would be produced in the CNS since transport of such a large protein (45,000 dal) across the blood-brain barrier is unlikely. Whether or not AGP stimulates the recapturing of 5-HT and thereby regulating neuronal disposition of this neurotransmitter remains unanswered.

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