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HUMAN SALIVARY EICOSANOIDS: CIRCADIAN VARIATION

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A circadian rhythm in the concentrations of prostaglandins (PG) E_2, PGF_2, PGI_2 (measured as 6-keto-$PGF_{1\alpha}$), immunoreactive hydroxyeicosatetraenoic acids and immunoreactive 6-sulfidopeptide containing leukotrienes in human mixed saliva was observed. The rhythm reflected changes in the absolute amounts of these compounds in saliva. Under usual sleep-wake cycles a single peak occurred during sleep with maximal levels at 5:00 AM; the amplitude of the peak varied for each product. The rhythm was sleep-dependent and a shift occurred when the sleep-wake cycles were displaced. Basal levels of these eicosanoids were maintained even without sleep.

Various metabolites of arachidonic acid, belonging to the group of twenty carbon fatty acid products referred to collectively as eicosanoids, have been identified in human mixed saliva. Products of both the cyclo-oxygenase [prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$, PGI_2 and thromboxane A_2 (TxA_2)] and lipoxygenase pathways [several hydroxyeicosatetraenoic acids (HETEs) and 6-sulfido-peptide containing leukotrienes (SRS)] have been detected. Their origin remains uncertain, but indirect evidence suggests that it may be in the salivary glands themselves (1). It has been speculated that salivary eicosanoids may participate in defense mechanisms in the oral cavity and absorption phenomena in the gastrointestinal tract (1). An interesting feature of their secretion is that the concentration of each compound in mixed saliva remains independent of salivary flow rate (2).

While assaying these compounds in human saliva it became evident that in a given individual, their concentration was affected by the time of sampling. The possibility of a time dependent variation of their concentrations in human saliva was investigated in the present study.

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METHODS

Mixed saliva samples were collected at specified times over a 24-hour period. Subjects (four from each sex) were 23 to 55 years old, non-smokers, apparently healthy, free of any oral or salivary gland disease and were receiving no medications. Saliva flow was stimulated by chewing on a teflon bolus and the accumulating saliva was collected in sterile tubes. The first 2 ml were discarded and the subsequent 3-5 ml were stored immediately in dry ice until transported to the laboratory where they were kept at -20°C (2). Eicosanoids were extracted from saliva as described (1, 2) and assayed by radioimmunoassay. The antisera against PGE_2, $PGF_{2\alpha}$, and 6-keto-$PGF_{1\alpha}$ are highly specific. The other two are class-specific; i.e., iHETE measures 12-HETE (homologous ligand), LTB_4, 5-HETE, 15-HETE (20%, 2-5%, and 2-5%, respective cross reactivities); iSRS measures LTC_4, LTD_4, LTE_4 and their 11-trans-stereoisomers (the antiserum displays comparable affinities to each) (1, 2).

RESULTS AND DISCUSSION

As shown in Fig. 1, the concentration of each of these products manifested a prominent peak with maximal levels at 5:00 AM. Concentrations started to increase around 10:30 PM and returned to plateau levels around 9:00 AM. Five of these subjects repeated the 24 hour saliva collection either on successive days or as far apart as 5 months, and this pattern of variation was satisfactorily reproduced in all five. Study of salivary eicosanoids in samples obtained by a slightly different collection method every 30 min for 5 hours from four adult males disclosed episodic elevations in their levels which appeared to be of a stochastic nature (data not shown). The ratio of the 5:00 AM peak level to the average plateau level was greatest for $PGF_{2\alpha}$ (4.0) and smallest for iHETEs (1.9); the ratios for the other products were between these two extremes. Since the concentrations of these eicosanoids varied independently of each other during the 24-hour period, the observed circadian variation in their concentrations must have been brought about by changes in their absolute amounts in saliva and not simply by production of more concentrated saliva. If the latter were true, then the relative ratio of the products to each other would have been relatively constant throughout the 24 hours; this was not observed. The period of the rhythm could not be determined with great accuracy since sampling was not frequent enough. It was close to 24 hours and in this sense the rhythm was a circadian one (3); actually, this could be called a diel rhythm as it was measured only in natural day-night cycles (4).

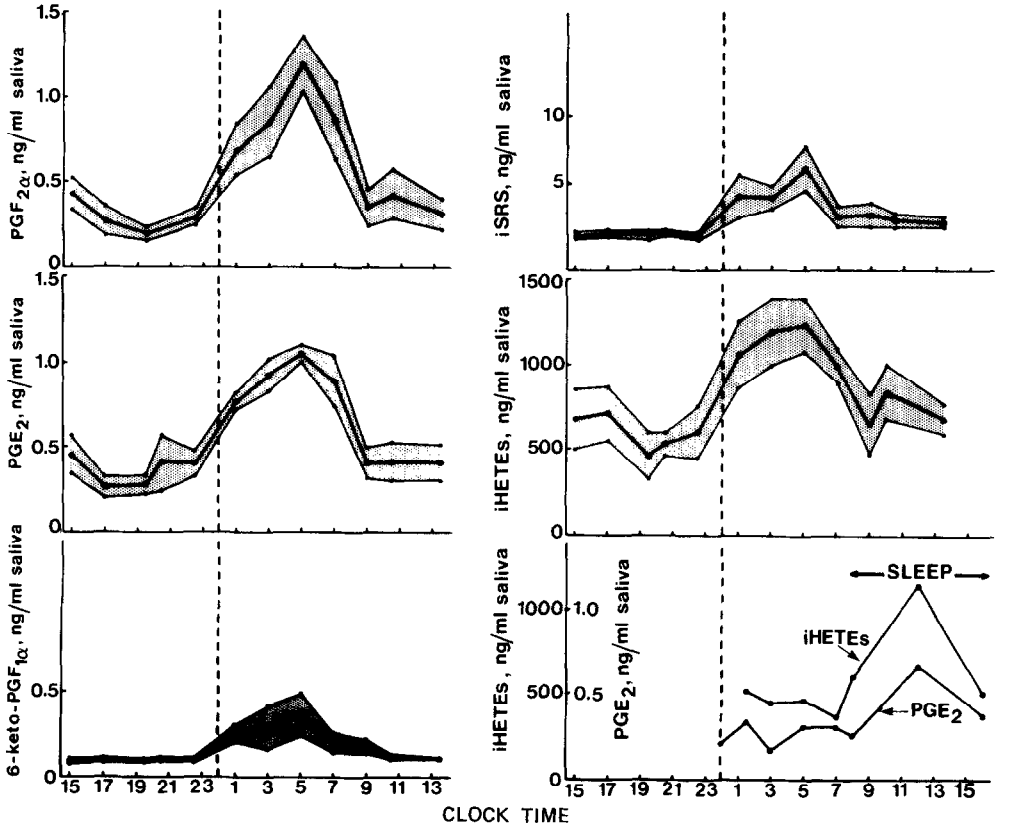

Fig. 1. Circadian rhythm in the concentrations of several eicosanoids in human mixed saliva. The shaded area indicates one SEM above and below the mean value (thicker line). The broken perpendicular lines indicate midnight. The last panel depicts the levels of PGE_2 and iHETEs in a night nurse; levels of the other three serologic activities, which followed the same pattern, are omitted for clarity.

Every subject maintained his/her daily activities unperturbed by the study. Review of the detailed activity diaries kept by each individual during saliva collection indicated lack of association between any activity and the eicosanoid levels in saliva. The eicosanoid peaks occurred, however, in all eight subjects during their sleep hours; all subjects reported going back to sleep promptly after saliva collection during the night. To investigate the effect of sleep on the periodicity of salivary eicosanoid levels we studied four night shift nurses whose sleep-wake cycles had been displaced for over a year. They were generally awake between 10 PM and 8 AM and slept during the morning and early afternoon. Every participating subject was working for at least four successive nights prior to saliva collection. Samples were collected every two hours between midnight and 8:00 AM while subjects were

working. During this time no eicosanoid peaks were observed in any of these nurses (data not shown). The last panel in Fig. 1 shows the shifted rhythm in the one of the nurses who, in addition, collected saliva during her period of daytime sleep. These data indicate a sleep-dependence of the rhythm.

The possibility that this circadian rhythm was entrained by corticosteroids was examined for the following reasons: (i) plasma corticosteroid concentrations display a circadian periodicity with peak concentrations occurring prior to, or at the time of, awakening with a decline over the remainder of the 24-hr period (5); (ii) steroids are potent inhibitors of arachidonic acid transformations (6-12); and (iii) salivary cortisol changes in tandem with plasma cortisol (13). Three adults in apparently excellent health collected saliva samples at approximately 2-hr intervals on two consecutive days. At the end of the first day, at 11 PM, each received 1 mg of dexamethasone orally, a dose known to suppress the morning peak of corticosteroids in healthy individuals (14). The rhythms of all five eicosanoids in these subjects remained typically circadian and showed essentially no change between the two days. This observation could be interpreted as either showing that salivary eicosanoid levels oscillate independently of corticosteroids or that their response to a "suppressed" corticosteroid periodicity takes place after a lag period. The possibility that dexamethasone failed to suppress the methasone orally, a dose known to suppress the morning peak of corticosteroids, cannot be ruled out but appears very unlikely. Of the other periodically secreted hormones which, owing to their oscillation frequencies, could entrain this rhythm (growth hormone, prolactin, aldosterone, testosterone and thyrotropin (3,4)) none, so far, is known to affect salivary eicosanoids.

The biological significance or the evolutionary advantage of this circadian rhythm in the levels of salivary eicosanoids is unknown, especially since their physiological role remains unclear. One may speculate that the increased concentration of eicosanoids during sleep compensates for the decreased salivary flow rate (15), so that teeth and other elements in the

oral cavity are well protected. Also, if HETEs, which represent by far the greatest fraction of salivary eicosanoids (1), do participate in absorption phenomena in the gastrointestinal lumen (16), their nocturnal outpouring may be part of a temporally ordered digestive process. Several steps in arachidonic acid metabolism could be influenced by the biological clock. The findings that levels of both lipoxygenase and cyclooxygenase products are increased during sleep suggest that the expression of acylhydrolase activities is responsible for the rhythm.

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