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EXPOSURE TO ANTARCTIC SUMMER AFFECTS THE IMMUNOGLOBULIN RHYTHM BUT NOT THE ORAL TEMPERATURE RHYTHM

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Abstract

Environmental light is an important zeitgeber in humans. The long polar days of Antarctica during the summer period can disrupt the internal clock thereby desynchronizing the various bodily rhythms, which can have health implications. Hence, in the present study, in one group of six volunteers, the immunoglobulin rhythm was assessed by four hourly blood sampling. In another group of six volunteers the oral temperature rhythm was measured. The results reveal disruption of the immunoglobulin rhythm due to the influence of exogenous components and possible involvement of neuroendocrine factors. However the oral temperature rhythm was unaffected during or after a short stay in Antarctica during the summer period. The importance of regular sleep-wake cycle and other social cues in maintaining a stable temperature rhythm is implicated.

Introduction

Extreme environmental conditions and psychosocial factors of living in isolation are potentially stressful for a human sojourner in Antarctica. Amongst the environmental conditions existing in Antarctica, the altered photoperiodicity is of particular interest from a chronobiological view. Since there is prolonged light exposure during summer in Antarctica, the human circadian rhythm is prone to undergo alterations and disruptions.

Biological rhythms play a definite role in maintaining a normal homeostasis of humans. Circadian rhythms in humans are a mixture of endogenous and exogenous components that are derived from the body clock and the interaction between our environment and lifestyle. Inherently, the body clock tends to run slow (by solar time) with a period of about 25 hours. Under normal circumstances, however, zeitgebers adjust it to run with a period of exactly 24 hours. Recent evidences show that the hypothalamic suprachiasmatic nuclei (SCN) is both necessary and sufficient for the generation of circadian rhythmicity in

mammals (Klein *et al,* 1991), though other sites, including the pineal gland, might also play some role. The important zeitgebers in humans appea to be a mixture of bright light and social factors. Orcadian rhythms not only enable us to adjust better to our rhythmic environment but also influence our responses to disease processes and drugs.

Circadian rhythm of various immunological parameters such as, total leukocyte count (Haus *et al.,* 1983), phagocytic cells (Knyzynski and Fischer, 1981), natural killer cell activity (Gatti *et al,* 1987), Lymphocyte proliferation (Tavadia *et al,* 1975) have been reported. In conditions of altered photoperiodicity, it is possible that there could be a disruption in the circadian rhythm of immune parameters, which could lead to an impairment of the immune function. Hence in the present study the circadian rhythm of oral temperature and immunoglobulins (IgG & IgM) was measured so as to assess whether the disruption of circadian parameters is associated with immune parameters.

Materials and Methods

Subjects

The study was conducted on two groups of volunteers, who were members of the summer team of the 14th Indian Scientific Expedition to Antarctica. In Group-I there were 6 subjects with a mean age of 35.5 ± 5.32 years. In these subjects the oral temperature rhythm was measured in three phases. In Group-II there were 6 subjects with mean age of 33.45 ± 3.48 years. Four hourly blood sampling was done on these subjects to estimate the circadian rhythm of IgG and IgM, in two phases before and after exposure to Antarctic summer.

Experimental Phases

The Oral temperature rhythm was recorded in three phases. First on board ship while in the warm waters, which forms the basal (23 °*T* S, 56°5' E, L/D 13:11, 24 ± 2 °C). Second, at summer camp of Maitri, after three weeks of Antarctica stay during the austral summer (70°5' S, 11 °4 E, L/D 23:01, -10.5 °C). Third, on board ship during the return voyage after the ship left Antarctic cold waters and moved into warmer latitudes (26°5' S, 54°6' E, L/D 13:11, 26 ± 3 °C).

The Immunoglobulin rhythm was assessed in two phases. First, on board ship, under basal conditions before exposure to Antarctic conditions (23° S, 56° E, L/D 13:11, 24 ± 2 °C). Second, on board ship during the return voyage, after exposure to Antarctic summer conditions (26° S, 54° E, L/D 13:11, 26 ± 3 °C).

Procedures

Temperature Rhythm,

Self-measurement of the oral temperature rhythm (autorhythmometry) was done using the clinical thermometer (Doctor, India). The readings were noted in two hourly intervals at every even hour. Self-measurements of oral temperature rhythm have been used by other workers also and have been found to be reliable(Pati and Gupta, 1994;Motohashi, 1990;Reinberg *et al*,, 1988). In these studies the sleep period measurements were not recorded, whereas in the present study, in order to include the sleep time values also the measurements were made by the investigator with only minimal disturbance to the subject.

Immunoglobulin Rhythm

Four hourly blood sampling was done for a period of 24 hours and the serum was separated. Blood samples were drawn at 1800, 2200, 0200, 0600, 1000 and 1400 hour's local time. The serum concentration of Immunoglobulin G and Immunoglobulin M were quantified by enzyme linked immunosorbant assay (ELISA) following the method of Kaur *et al.* (1991).

Enzyme Linked Immunosorbant Assay (ELISA) of Immunoglobulins

The standard Immunoglobulin G or Immunoglobulin M (DAKO) or the test sera optimally diluted in 0.5 M Carbonate Bicarbonate Buffer (pH 9.6) were coated in triplicate wells on to the solid phase (COSTAR ELISA plates, 96 well, flat bottom) and incubated at 37 °C for 4 hours in'a humid chamber. After decanting and washing with Phosphate Buffered Saline (PBS 0.1 M; pH 7.25), non specific binding was blocked using skimmed milk powder (Anikspray) by incubating at 37 °C forone hour. Following PBS-Tween washing Anti Human Ig-HRP conjugate (DAKO) was then added and incubated for one hour at 37 °C. The chromogen (Ortho Phenylene diamine dihydrochloride [OPD], Sigma) dissolved in the Substrate buffer (1.5 M citrate phosphate buffer; pH 5.0) was used to develop the color and the reaction was stopped using 8N H_2SO_4. The optical density (OD) was then read in an ELISA reader, (Pharmacia) at 490 nm.

Statistical Analysis

The circadian rhythm data were analyzed using the cosinar analysis (Nelson *et al,* 1979) to obtain the circadian.parameters viz., Percentage rhythm (PR), Mesor (M) the rhythm adjusted mean; Amplitude (AMP) half the total variability between peak and trough; Acrophase (ACRO), peak time with mid-night as arbitrary phase reference. Each rhythm was individually analyzed

and the resultant circadian parameters were pooled and their mean was obtained. Paired 't' test was used to find out the differences between two group means.

Results

IgG Rhythm

The IgG rhythm was unaltered in terms of the percentage rhythm and acrophase, however there was a significant decrease in the mesor ($p<0.001$) and amplitude ($p<0.05$) after Antarctic exposure (Table-1).

Table 1:: Circadian Rhythm of IgG

Parameters	Basal	After Summer
PR (%)	52.57 ±22.58	48.79 ±25.64
MESOR (mg/dL)	1514.84 ±113.73	764.50 ± 43.78 a3
AMP (mg/dL)	191.04±100.45	90.35 ± 30.95al
ACRO (hrs)	22.63 ± 5.07	22.17 ±3.92

a = In comparison with basal; l=$p<0.05$, 2=$p<0.01$, 3=$p<0.001$

IgM Rhythm

The IgM rhythm (Table-2) also was unaltered as seen by the stable percentage rhythm, however phase advancement in the timing of the acrophase was noticed after Antarctic exposure. No changes in the mesor or amplitude were noticed in the IgM rhythm.

Table 2: Circadian Rhythm of IgM

Parameters	Basal	After Summer
PR (%)	57.44 ±16.17	42.99±20.58
MESOR (mg/dL)	112.29 ±12.75	94.80 ±18.69
AMP (mg/dL)	4.68 ±2.67	6.93 ± 3.93
ACRO (hrs)	21.34 ±2.79	18.77 ±6.39

Oral Temperature Rhythm

The oral temperature rhythm was not disrupted during or after Antarctic exposure **(Table-3).** A minimum of 50% of the subjects showed a significant rhythm in all the phases studied. The timing of the acrophase also remained stable without showing any significant changes.

Table 3: Temperature Rhythm

Parameters	Basal	Summer	Return
PR (%)	41.84 ±22.50	49.85 ± 20.89	57.82 ±21.32
M(°C)	36.39 ±0.39	36.28 ±0.42	36.57 ±0.22
AMP (°C)	0.27 ±0.16	0.30 ± 0.22	0.36 ±0.16
ACRO (hrs)	16.67 ±4.19	15.31±7.83	20.84 ±6.15

Discussion

After exposure to Antarctica there was a significant decrease in the amplitude and mesor of IgG rhythm without any changes in the percentage rhythm or acrophase. The unchanged percentage rhythm indicates that the rhythm continues, albeit with a diminished amplitude. This indicates that the endogenous component of the rhythm is unaffected. The decreased amplitude and mesor. of the rhythm, the two parameters influenced by the exogenous component, clearly indicates that the exogenous component for the generation of IgG rhythm is affected (Redfern *et al,* 1991). These changes in the immunoglobulin rhythm being present during the return voyage, after exposure to Antarctic summer, indicates the possible involvement of neuroendocrine factors. It is interesting to note that only IgG and not IgM rhythm was affected.

Body temperature rhythm of subjects under normal photoperiodicity, during the onward voyage was circadian with peak in late evening hours. This is consistent with earlier reports (Pati and Saini, 1991; Motohashi *et al.,* 1987). Gander *et al.* (1991) reported, in three subjects, a phase delay on temperature rhythm associated with sleep disturbances in Antarctic summer. However, in the present study there was no such phase delay in the acrophase of temperature rhythm during their stay in Antarctica. But during the return voyage there was an insignificant phase delay in the time of the acrophase without any other changes in the circadian rhythm. Humans 'free run' when in temporal isolation (Chandrashekaran *et al,* 1997; Kennaway and Van-Dorp, 1991) exhibiting unentrained circadian rhythms, indicating the importance of environmental light as a zeitgeber However the body temperature and thyrotropin rhythm in humans are partly under pacemaker control and partly secondary to the rhythmic superimposition of sleep (Wever, 1985; Parker *et al,* 1987). Hence in the present study the unaltered temperature rhythm observed even in conditions of exposure to prolonged natural light might have been due to the entrainment by the sleep-wake cycle and by social cues (timing of food, work hours etc.).

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