Circadian organization of serum electrolytes in physiological aging

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Summary

Age-related structural and neurochemical changes occurring in the central nervous system have been related to changes in some rhythmometric parameters. In spite of their clinical importance, only a few studies have investigated the modifications over time of serum electrolytes in senescence. The aim of our study was to evaluate the circadian pattern of serum potassium, chloride, sodium, calcium and phosphorus in 30 clinically healthy elderly subjects, with no cognitive impairment, and to compare the findings with those given by 24 healthy young controls. Both the young and the elderly subjects exhibited statistically significant circadian rhythms for all serum electrolytes considered. Our findings suggest that circadian organization of serum electrolytes is maintained in physiological aging, even though it should be noted that sodium and phosphorus acrophases differed significantly in the two experimental groups.

KEY WORDS: aging, circadian rhythm, elderly, electrolyte.

Introduction

It has been suggested that the 24-hour time structure influences a wide range of physiological, serological and urinary variables in humans (1). Recent studies have demonstrated, in both young (1,2) and old people (1,3,4), the importance of circadian organization, both from a pathophysiological and a pharmacological (1) point of view. Altered rhythms are presumably involved in processes of cell and organ damage, and they have been suggested to play a role in the aging process in humans (4). In fact, neuroendocrine system impairment and a decline of hormonal cyclicity, both due to anatomical damage and neurotransmitter imbalances in the central nervous system (CNS), have recently been reported in physiological aging (4,5). All these alterations present in aging may be attributable to a derangement of the circadian system. The suprachiasmatic nucleus seems to be one of the main structures governing and maintaining the temporal organization of the human organism. Given this background, senescence and brain aging are often claimed to be related to a decrease in the capacity of various functions of the organism to adapt to environmental modifications (4). These alterations, associated with aging, may be exaggerated in pathological aging and contribute to senility and cognitive impairment (4-6). Thus, subjects who achieve very long life-spans could constitute a suitable model for investigation of the biological basis of physiological aging. In spite of their clinical importance, only a few studies have investigated the modifications over time of serum electrolytes in senescence. In fact, age-related changes in the neuroendocrine control of salt and water balance have been suggested to predispose elderly people to fluid and electrolyte alterations, such as hyper- and hyponatriemia, especially in conditions of physiological stress or in the course of common illnesses (7). We therefore set out to evaluate the circadian rhythmicity of several clinically important serum electrolytes (potassium, chloride, sodium, calcium, and phosphorus) in a group of elderly subjects, with no cognitive impairment, comparing the findings with those obtained in a control group of healthy young people.

Materials and methods

Subjects

The study was carried out, in the spring of the years 1996 to 2000, at the Department of Internal Medicine and Medical Therapy, University of Pavia, Italy (Pavia has a latitude of 45°11’ N, a longitude of 9°8’ O; and an altitude of 77 m above sea level). Thirty healthy elderly subjects (18 females and 12 males, mean age±SD 87±6 years) were studied after hospital admission for clinical check-ups. The control group consisted of 24 healthy young controls (14 females and 10 males, mean age 29±3). The elderly and young subjects gave their written
consent to the study and the protocol had the approval of the Ethics Committee of the Department of Internal Medicine of the University of Pavia. To synchronize all the participating subjects to the same dietary and daily schedules, none of the elderly subjects or the controls were studied sooner than ten days following their admission to hospital. The inclusion criteria for the elderly subjects were: age 75 years or over, normal arterial blood pressure levels, no prescribed medication, and no known serious medical diagnoses (debilitating or chronic diseases) based on the criteria used in the SENIEUR Protocol (8). All the subjects had a normal body mass index (between 18 and 28 kg/m$^2$) and presented no alterations of consciousness (Mini Mental State Examination > 24) (9) or of the sleep/wake cycle, or major depression, evaluated by the Hamilton test (10). All the participating subjects, both elderly and young, had three meals daily: breakfast between 07.00 and 08.00, lunch between 12.00 and 13.00, and dinner between 18.00 and 19.00. The subjects followed a regular hospital diet, and the food was provided by the hospital kitchen. No drugs had been administered for at least 15 days. The intake of alcoholic beverages was avoided.

Daytime was spent in sedentary activities (television, reading, socializing, etc.). Nocturnal rest in darkness was from 22.00 to 06.00. The first blood sample was collected at 08.00; subsequent ones were collected every 4 h thereafter until 20.00 and every 2 h from 20.00 to 04.00. This frequency of sampling has been demonstrated to be sufficient to allow monitoring of the circadian periodicity of a function (11). Serum and plasma were immediately separated and frozen at -80°C until the assay.

**Laboratory procedures**

The quantitative determination of sodium, potassium and chloride in serum was performed by means of an ion-selective electrode (ISE) method, using an Integra 700 (Roche Diagnostics, Basel, Switzerland). The measurement of serum calcium was performed by means of an automated colorimetric assay (again using a Roche Integra 700), based on the reaction of calcium with o-cresophthalein in alkaline solution, leading to the formation of a calcium-o-cresophthalein complex. Serum levels of phosphorus were also measured using a Roche Integra 700, by means of a method based on the reaction of phosphorus with ammonium molybdate to form ammonium phosphomolybdate without reduction.

**Statistical analysis**

The results were expressed as mean values±SD. Student’s t-test was used to assess the significance of differences between patients and healthy subjects. Rhythmic data were analyzed by single and population mean cosinor analysis according to Halberg (12); the comparison of the rhythm parameters of patients and controls was carried out using the mesor test and the amplitude-acrophase Hotelling test, according to Bingham et al. (13). The cosinor method provided the probability of rejection of the null hypothesis for a chosen period (i.e., 24 h) and the rhythm characteristics, with their 95% confidence intervals. These characteristics were the mesor (24-hour-adjusted means), the amplitude (half the difference between the maximum and the minimum fitted cosine function), and the acrophase (time of maximum in fitted cosine function, with midnight as the phase reference). An analysis using chronograms (mean±SD) was employed. P values of less than 0.05 were considered significant.

**Results**

**Potassium**

Figure 1 shows the circadian profile of serum potassium in the elderly and young subjects. As set out in Table I, population-mean cosinor analysis revealed that the circadian rhythm of potassium reached statistical significance both in the elderly subjects (p<0.001) and in the young subjects (p<0.018). The mesor was significantly higher in the elderly group than in the controls (p<0.040). The acrophase occurred later in the elderly subjects (16:53 h versus 13:21 h in the young controls).

**Chloride**

Figure 2 shows the 24-hour profile of serum chloride in the elderly and in young subjects. As shown by the

![Figure 1 - Circadian pattern of potassium (mean±SD) evaluated in 30 healthy elderly subjects (triangles) and in 24 young controls (circles).](image1)

![Figure 2 - Circadian pattern of chloride (mean±SD) evaluated in 30 healthy elderly subjects (triangles) and in 24 young controls (circles).](image2)
population-mean cosinor analysis (Table I), a statistically significant chloride circadian rhythm was detectable both in the elderly (p<0.0006) and in the control group (p<0.0013). The mesor and the amplitude of chloride circadian fluctuations were not significantly different in the elderly versus the young subjects. The acrophase appeared at 01:40 in the elderly group and at 01:19 in the young controls.

**Sodium**

Figure 3 shows the circadian profile of plasma sodium in the two sets of subjects. As population-mean cosinor analysis (Table I) showed, a statistically significant sodium circadian rhythm was detectable both in the elderly (p<0.05) and the young group (p<0.05). The mesor of sodium fluctuations was not significantly different in the elderly subjects versus the controls. A significant reduction of the amplitude of sodium circadian fluctuations was reported in the elderly subjects (p=0.018636). The acrophase occurred at 04:40 in the elderly subjects and at 14:49 in the young controls.

**Calcium**

Figure 4 shows the circadian profile of plasma calcium in the elderly and in the young subjects. As shown in Table I, the circadian rhythm of calcium reached statistical significance both in the elderly subjects (p<0.0001) and in the young controls (p<0.008). The mesor and the amplitude of calcium circadian fluctuations were not significantly different in the elderly compared with the young subjects. The acrophase appeared at 12:38 in the elderly group and at 15:58 in the controls.

**Table I - Population-mean cosinor analysis of the parameters evaluated in the study.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>p</th>
<th>Mesor (mean±SE)</th>
<th>Amplitude (mean±SE)</th>
<th>Acrophase (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium</td>
<td>Young subjects</td>
<td>0.018</td>
<td>3.66±0.14</td>
<td>0.10±0.03</td>
</tr>
<tr>
<td></td>
<td>Elderly subjects</td>
<td>0.001</td>
<td>4.37±0.07</td>
<td>0.19±0.03</td>
</tr>
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<td>Chloride</td>
<td>Young subjects</td>
<td>0.0013</td>
<td>104.45±0.59</td>
<td>1.20±0.70</td>
</tr>
<tr>
<td></td>
<td>Elderly subjects</td>
<td>0.0006</td>
<td>105.29±0.92</td>
<td>1.52±0.21</td>
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<tr>
<td>Sodium</td>
<td>Young subjects</td>
<td>0.050</td>
<td>141.42±1.42</td>
<td>1.41±0.50</td>
</tr>
<tr>
<td></td>
<td>Elderly subjects</td>
<td>0.050</td>
<td>143.11±0.86</td>
<td>0.59±0.17</td>
</tr>
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<td>Calcium</td>
<td>Young subjects</td>
<td>0.008</td>
<td>2.37±0.02</td>
<td>0.25±0.07</td>
</tr>
<tr>
<td></td>
<td>Elderly subjects</td>
<td>0.000</td>
<td>2.33±0.03</td>
<td>0.19±0.03</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>Young subjects</td>
<td>0.0001</td>
<td>1.37±0.04</td>
<td>0.38±0.08</td>
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<tr>
<td></td>
<td>Elderly subjects</td>
<td>0.025</td>
<td>1.12±0.06</td>
<td>0.04±0.01</td>
</tr>
</tbody>
</table>

* Phase reference: local midnight; 360° = 24 h; 15° = 1 h
Abbreviations: SE = standard error; CI = confidence interval
Phosphorus

Figure 5 shows the 24-hour profile of serum phosphorus in the elderly and in the young subjects (at each time point, the asterisks show the significant difference between the two groups. Student’s t test: *p<0.05). Population-mean cosinor analysis (Table I) revealed a statistically significant circadian rhythm both in the elderly (p<0.0001) and in the young (p<0.025) groups. A significant reduction of the amplitude of phosphorus circadian fluctuations was reported in the elderly subjects (p<0.010133). The acrophase occurred earlier in elderly group (21:53 versus 02:38 in the young controls).

Discussion

Our results showed that the circadian organization of serum electrolytes is maintained in physiological aging, in spite of the emergence, in senescence, of changes in some rhythmometric parameters (3-6,14,16) – a consequence of the organism’s reduced plasticity in the face of daily and seasonal environmental modifications (4). These changes, present in aging, may be exaggerated in pathological aging and could contribute to the generation of senile dementia (5,6). Our findings are largely in agreement with those reported by Haus (1), who also investigated the circadian rhythmicity of several metabolic and endocrine parameters in young and elderly subjects from Romania, even though it should be noted that direct comparison of studies is quite difficult and could result in conflicting data. In fact, the human time structure is not necessarily the same in subjects living under different environmental conditions and in different geographical locations. Furthermore, as a consequence of their past history and lifestyle, or of a disease or treatment, some subjects may show a different phase relation and/or changes in amplitude with respect to the usually dominant environmental synchronizers. As previously suggested (4), the amplitude of certain circadian periodic variables is regarded as the most prominent index of aging and constitutes a measurable end-point for determining the stage and progression of the same. In particular, the decrease in the circadian amplitude appears to be an important part of the aging process and could be related to a “flattening” of the circadian pattern in old age (4-6, 14).

In accordance with this, we found a decreased amplitude of phosphorus and sodium circadian fluctuations in the elderly group, as well as a phase advance in phosphorus and calcium nycthemeral rhythms. The latter is another temporal characteristic reported in the elderly and may contribute to the establishment of an internal desynchronization of periodic functions (4). Nevertheless, the persistence of the circadian time structure here reported strongly suggests that the circadian clock is still efficient in clinically healthy elderly people, but presents alterations that can provoke rhythm disturbances (4).

In conclusion, our findings seem to confirm the maintenance, when cognitive impairment is absent, of the circadian time organization of serum electrolytes in physiological aging. This suggests that electrolytes might also play a role as indices of CNS activity, as recently also indicated in a study showing a relationship between serum sodium and chloride concentrations and psychometric test scores (17). The uncovering of the circadian pattern of these parameters could also provide some useful pointers for the management and the chronopharmacology of drug administration in certain diseases related to alterations of serum electrolytes (1).

References

Electrolyte circadian rhythms in the elderly


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