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Erythrocyte Magnesium and Prostaglandin Dynamics in Chronic Sleep Deprivation

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Summary

Background and hypothesis: The mechanism of sudden cardiac death occurring in patients with chronic fatigue is controversial. This study was designed to define a hypothesis that coronary arterial spasm and thrombus formation can occur during chronic fatigue.

Methods: For evaluating the feasibility of coronary arterial spasm, erythrocyte magnesium (Mg) was measured. Blood coagulability was evaluated by the change of prostaglandin concentration. Subjects included 16 healthy male volunteers (mean age 21.6 ± 2.5 years). Test conditions were as follows: (A) control state: a day following a night of good sleep; (B) temporary sleep deprivation: a day preceded by < 3 h of sleep; (C) chronic sleep deprivation: a day preceded by a month during which sleep lasted < 60% of that in condition (A) above. The erythrocyte Mg concentration was measured by the atomic absorption method. The plasma concentration of thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ were measured in eight subjects by radioimmunoassay method.

Results: (1) Mean erythrocyte Mg concentration was significantly less in chronic sleep deprivation (1.1 ± 0.4 mg/dl) than in the control state (1.8 ± 0.4 mg/dl, $p<0.01$) or in temporary sleep deprivation (1.6 ± 0.4, $p<0.01$). (2) The level of thromboxane B_2 was significantly higher during chronic sleep deprivation than under control conditions (104.4 ± 78.0 vs. 20.4 ± 9.0 pg/ml, $p<0.05$). (3) There were no significant intergroup differences in 6-keto-prostaglandin $F_{1\alpha}$ level.

Conclusion: These findings could support the hypothesis that coronary arterial spasm and thrombus formation occur in chronic sleep deprivation.

Key words: chronic sleep deprivation, erythrocyte magnesium, thromboxane B_2, 6-keto-prostaglandin $F_{1\alpha}$

Introduction

Sudden cardiac death has been reported to be the most frequent among the sudden deaths.[1] Nearly 400,000 persons die suddenly in the United States every year,[2] and many sudden deaths occur in persons in apparently good health without any prior evidence of coronary heart disease.[3] Although precise epidemiologic reports are rare in Japan, Matoba *et al.*[4] reported that 810 of 1,230 autopsied cases of sudden death were of cardiac origin including coronary heart disease (77%), cardiomyopathy (7%), and valvular disease (3%). Sudden cardiac death has been reported to occur in apparently healthy persons in the state of chronic fatigue;[5] however, its mechanism is controversial.

We investigated the various influences of chronic sleep deprivation as a model of chronic fatigue on stress hormone secretion including catecholamines, ACTH, and cortisol.[6] That study demonstrated the tendancy for decreased secretion of catecholamines and cortisol during chronic sleep deprivation compared with the control state or temporary sleep deprivation. In the present study, attention has been directed to the dynamics of magnesium (Mg) and prostaglandin (PG) because many patients with vasospastic angina, which is suggested to be an important etiology of sudden cardiac death, exist in Japan.

The purpose of the present study was to investigate whether Mg and PG dynamics could be changed. This study was designed based on the clinical background that coronary arterial spasm and thrombus formation could occur in the state of chronic fatigue. For evaluating the feasibility of coronary arterial spasm, erythrocyte Mg concentration was measured because it reflects the bodily Mg content. Blood coagulability was evaluated by the change of PG dynamics.

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Subjects and Methods

Sixteen apparently healthy male volunteers (college students aged 21.6 ± 2.5 years) were studied. Test conditions were defined as follows: (1) control state: a day following a night of good sleep; (2) temporary sleep deprivation: a day preceded by <3 h of sleep due to study for an examination; and (3) chronic sleep deprivation: a day preceded by a month during which sleep lasted <60% of that in condition (A) above because of study for an examination. No restrictions other than alcohol ingestion, which has influence on Mg excretion, were imposed on the daily life of the subjects. Hours of sleep of each state were investigated by the interview questionnaire. Informed consent for participation was obtained from each subject before the study.

Measurement of Erythrocyte Mg Concentration

Samples of heparinized blood for measuring erythrocyte Mg concentration were centrifuged at 3000 × G at 4°C for 10 min. After removal of plasma, the erythrocyte sediment was washed three times with 9% NaCl solution and centrifuged at 4°C at 3000 × G for 10 min. After removal of the supernatant, 9% NaCl solution was added until the total sample volume reached 4 ml; this volume was divided equally into two samples. Part of a sample was used to count erythrocytes. Distilled water was added to the remaining part of that sample to hemolyze the erythrocytes. Erythrocyte Mg concentration was measured by the atomic absorption method and the value obtained was corrected for the number of erythrocytes; Mg concentration was expressed per 400 × 10^4/mm^3.

Measurement of PG Concentration

This study was performed in eight subjects. Blood 7 ml was aspirated into a polypropylene tube containing 4.5 mM EDTA, indomethacin, and tradirol for measuring thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$. Samples were centrifuged at 3000 × G for 10 min at 4°C to produce platelet-poor plasma for use in a prostanoid assay. Thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ were measured by radioimmunoassay.

Values are expressed as mean value ± standard deviation. Differences between groups were evaluated by Fisher's PLSD method after an initial evaluation by one-way analysis of variance. A p value of <0.05 was considered statistically significant.

Results

Change in Erythrocyte Mg Concentration Caused by Sleep Deprivation (Fig. 1)

The mean erythrocyte Mg concentration was significantly less in the state of temporary sleep deprivation (1.6 ± 0.4 mg/dl) than in the control (1.8 ± 0.4 mg/dl, $p<0.05$). The concentration in chronic sleep deprivation (1.1 ± 0.4 mg/dl) was significantly less than in the control state ($p<0.01$) and also in the temporary sleep deprivation ($p<0.01$).

Change in PG Concentration Caused by Sleep Deprivation (Fig. 2)

The mean plasma level of thromboxane B_2 was significantly higher in the state of chronic sleep deprivation (104.4 ± 78.0 pg/ml) than in the control state (20.4 ± 9.0 pg/ml, $p<0.05$). The plasma level during chronic sleep deprivation was also higher than during temporary sleep deprivation (17.7 ± 10.3 pg/ml), but this difference was not significant. There was no significant difference in 6-keto-prostaglandin $F_{1\alpha}$ level between the control state (11.2 ± 4.5 pg/ml) and the temporary sleep deprivation (10.5 ± 6.5 pg/ml) or the chronic sleep deprivation (10.9 ± 3.8 pg/ml) states. The mean value of the thromboxane B_2/6-keto-prostaglandin $F_{1\alpha}$ ratio was significantly higher in the

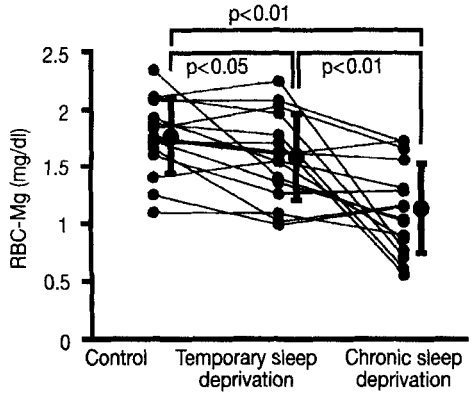

FIG. 1 Erythrocyte magnesium concentration in each condition. Continuous lines are presented as mean ± standard deviation. Mean erythrocyte magnesium concentration was significantly less in chronic sleep deprivation than in control state and also in temporary sleep deprivation. RBC-Mg: erythrocyte magnesium concentration.

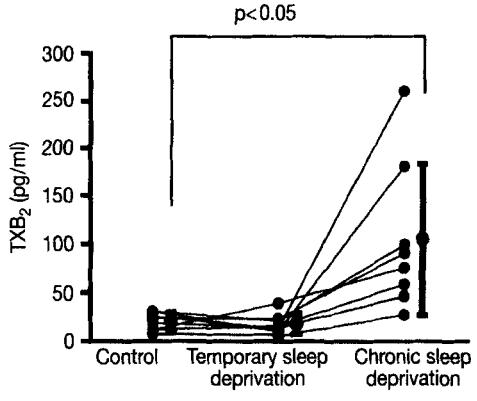

FIG. 2 Thromboxane B_2 concentration in each condition. Continuous lines are presented as mean ± standard deviation. The level of thromboxane B_2 was significantly higher in chronic sleep deprivation than in the control state. TXB_2: thromboxane B_2.

state of chronic sleep deprivation (9.5 ± 6.2) than in the control state (1.8 ± 0.5, $p<0.01$). Again, this ratio was higher in chronic sleep deprivation than in temporary sleep deprivation (1.9 ± 1.0), but this difference was not significant.

Discussion

Changes of Erythrocyte Mg Concentration in Chronic Sleep Deprivation

The mechanism of sudden cardiac death in chronically fatigued subjects is controversial both socially and medically. Epidemiologic studies suggest that myocardial hypomagnesemia may predispose to sudden cardiac death.[7] Also, death from ischemic heart disease is inversely correlated with Mg intake.[8] Several studies have shown that Mg content was decreased in the infarcted portion of the myocardium compared with noninfarcted segments.[9, 10] These findings support the hypothesis that myocardial Mg deficiency can predispose to sudden death due to ischemic heart disease. In vitro studies show that Mg deficiency induces constriction of isolated canine coronary arteries and that repletion of Mg dilates them.[11] We have reported that mean erythrocyte magnesium content is lower in patients with variant angina than in control patients and that its value was lower in patients with more frequent episodes of angina.[12] We have also demonstrated that measurement of erythrocyte Mg concentration is useful for determining the risk of vasospasm in patients with variant angina.[13] Thus, coronary arterial spasm may be related to Mg dynamics in the coronary arteries. From this background, the present study was undertaken to determine whether Mg dynamics may be affected by chronic fatigue.

The quantitative evaluation of chronic fatigue is difficult because of individual variations of the sense of fatigue. Toyoshima *et al.*[14] reported an epidemiologic study on relationships between mental stress and sudden cardiac death by obtaining information through the questionnaires from the bereaved family. In that study, the combination of mental stress and sleep deprivation may be a factor in the occurrence of sudden cardiac death in individuals under 60 years of age. Thus, we used chronic sleep deprivation as a model of chronic fatigue in the present study, and also because of the ease with which it can be replicated.

We used erythrocyte Mg concentration as an index of total body Mg content. Only about 1% of total body Mg is in the extracellular space, therefore assessments of the Mg content of muscle, lymphocytes, and erythrocytes, and measurement of daily urinary Mg output have been used as indices of the occurrence of Mg deficiency. Magnesium content for muscle may well be the best gauge of total body Mg stores, but it is an impractical determination in the usual clinical setting. Thus, we chose erythrocyte Mg concentration as an index of total body Mg content.

In the present study, we examined changes in Mg concentration caused by sleep deprivation on the assumption that Mg dynamics are involved in sudden cardiac death occurring in the chronic fatigued state. In subjects in a state of chronic sleep deprivation, mean erythrocyte Mg concentration was significantly lower. As already postulated, chronic sleep deprivation may lead to coronary arterial spasm through the hypomagnesemic state, which sets the stage for the occurrence of coronary events that ultimately result in acute myocardial infarction or anginal attack.

Concerning the mechanism of the diminution of erythrocyte Mg concentration, the repeated stress induced by chronic sleep deprivation may be a factor. Such stress, by increasing catecholamine secretion, would be expected to liberate free fatty acids in the blood.[15] Altura and Altura[16] suggested that an apparent Mg-deficient state might result from two major events: (1) the formation of insoluble salts via chelation of Mg with free fatty acids, and (2) the excretion of Mg via the kidneys because of overproduction of catecholamines. Henrotte *et al.*[17] reported that type A subjects had lower levels of erythrocyte Mg and also serum Mg than did type B subjects. In the present study, erythrocyte Mg concentration was already less in the temporary sleep deprivation than in the control state. Furthermore, in chronic sleep deprivation it was decreased still more. During chronic sleep deprivation, repeated stress such as temporary sleep deprivation would further reduce the erythrocyte Mg concentration. Over time, chronic repeated stressful episodes would provoke Mg deficiency, leading to an increase in the basal tone of the coronary arteries.

Prostaglandin Dynamics in Chronic Sleep Deprivation

Ellis *et al.*[18] suggested that thromboxane A_2 is involved in the contraction of coronary smooth muscle. Several studies have shown that prostanoids are important in modulating the coronary circulation.[19] Numano *et al.*[20] investigated plasma levels of thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ in patients with variant angina and measured the changes in these levels during the occurrence of coronary spasm. They reported that high levels of thromboxane B_2 in patients with atherosclerotic coronaries may be one factor that leads to coronary arterial spasm, and that low levels of prostacyclin may also contribute to such spasm; thus, any alteration in the balance between thromboxane A_2 and prostacyclin would lead to coronary heart disease. In the present study, the fact that the thromboxane B_2 level and the thromboxane B_2/6-keto prostaglandin $F_{1\alpha}$ ratio were higher during chronic sleep deprivation than during control conditions suggested that chronic sleep deprivation could produce coronary arterial spasm or coronary arterial thrombus.

These findings support the hypothesis that decrease of erythrocyte Mg content and increase of thromboxane B_2 in chronic sleep deprivation could predispose to cardiac events by inducing coronary arterial spasm or coronary arterial thrombus formation.

Conclusion

Chronic sleep deprivation leads to a cellular Mg deficiency and an increase in the level of thromboxane B_2 not associated

with an increase in 6-keto-prostaglandin $F_{1\alpha}$. These results support the hypothesis that coronary arterial spasm and thrombus formation could occur in chronic sleep deprivation.

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