Seasonal variations in cyclic GMP response on whole-body cooling in women with primary Raynaud’s phenomenon

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(Received 29 October 1996/7 March 1997; accepted 9 April 1997)

1. Primary Raynaud’s phenomenon (PRP) is characterized by increased vasoconstrictor tone that develops during exposure to cold. The symptoms are most pronounced during the winter months with low outdoor temperature. The L-arginine–nitric oxide (NO)–cyclic GMP (cGMP) pathway plays an important role in counteracting vasospasm. The aim of the present study was to investigate if the venous cGMP response to whole-body cooling in women with PRP varied with the season of the year.

2. The study was performed as an open parallel-group comparison between women with PRP and healthy female controls during the winter months of February 1994 and 1995 and in the summer month of August 1994. Blood samples were drawn just before and 40 min after whole-body cooling.

3. There were no significant changes in venous cGMP after whole-body cooling in women with PRP during the winter months of February 1994 and 1995. Cold exposure in the summer month of August resulted, however, in a significant increase in venous cGMP (P < 0.01). In contrast, the healthy women responded with a significant increase in venous cGMP on all three test occasions: February 1994 (P < 0.05), August 1994 (P < 0.05) and February 1995 (P < 0.01).

4. A seasonal variation in venous cGMP response to whole-body cooling was observed only in women with PRP. Healthy women responded to cold exposure with an increase in venous cGMP during summer and winter, whereas females with PRP showed an increase only during summer. Results from the present study might indicate seasonal variation in the regulation of constitutive nitric oxide synthetase in women with PRP, which may contribute to new therapeutic approaches.

INTRODUCTION

Primary Raynaud’s phenomenon (PRP) is a common complaint with a prevalence of approximately 15% in the Swedish female population aged 18–59 years [1]. The aetiology is unknown, but one possible cause, as originally proposed by Raynaud, is an increased vasoconstrictor tone, developing during exposure to cold and/or emotional stress [2]. In 1929, Lewis [3] suggested that the primary factor in Raynaud’s phenomenon was an abnormal sensitivity of the digital artery to direct stimuli, in particular low temperature, and referred to this defect as a ‘local fault’ [3].

Cutaneous vascular tone, which was considered to be solely under α-1-adrenergic vasoconstrictor control, with vasoconstriction as a reflex event, is now known to be controlled by neural and biochemical mechanisms in which the endothelium is an active participant. Recently published studies have identified novel endothelium-derived mediators of vascular tone, including the vasoconstrictor endothelin-1 and the vasodilator nitric oxide (NO) [4].

Studies during the last few years have increased our knowledge regarding the mechanisms underlying the relaxation of smooth muscle [5]. L-Arginine–NO–cyclic GMP (cGMP) plays an important role in counteracting vasospasm [6]. The mechanism is as follows: NO promotes an increase in cGMP, intracellular calcium decreases, which initiates the contractile elements of the smooth muscle cells to relax [7, 8]. As a result, vascular dilatation develops. In a recently published study [9], we found that a group of women with PRP, examined during the winter season, had no increase in venous cGMP during cold exposure.

Other factors of importance in the pathophysiology of PRP are neuropeptides such as calcitonin...

Key words: cyclic GMP, primary Raynaud’s phenomenon, seasonal variation.

Abbreviations: cGMP, cyclic GMP; CI, confidence interval; NO, nitric oxide; PRP, primary Raynaud’s phenomenon.

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gene-related peptide, calcium and magnesium ions [10]. Although complaints in patients with PRP are most pronounced during winter months, there are few studies where seasonal variation of pathophysiological factors have been investigated. In the study with women with PRP, seasonal variation in erythrocyte magnesium has been shown, with the lowest values in winter [11]. On the other hand there are a large number of studies where seasonal variation has been observed in factors such as blood pressure, serum lipids and fibrinogen, which might explain the higher mortality of cardiovascular disease during the winter season [12, 13].

In the present study, we investigated whether venous cGMP-response on whole-body cooling varied with the seasons of the year in women with PRP.

**METHOD**

**Subjects**

The Raynaud group comprised 24, 20 and 21 females in February 1994, August 1994 and February 1995 respectively, and were recruited from a population of women with PRP [1]. They had had pronounced PRP symptoms, both winter and summer, for a mean period of 17 years (range 2–40 years). The diagnosis was made by one investigator according to Allen and Brown’s diagnostic criteria [14]. The control group in each study consisted of 21, 21 and 25 healthy women respectively, recruited from the hospital staff, who did not have a previous history of PRP. Neither the women with PRP nor any of the controls had been exposed to vibrating tools or had a history of rheumatic illness. They had no symptoms or signs of ischaemic heart disease nor were they on any kind of drug treatment during the last 2 weeks before the investigation. Thirty-two of the females participated in all three studies, and seven participated in two of the studies. The clinical characteristics of the study participants, including the number of smokers and post-menopausal women, are given in Table 1. The study complied with the code of ethics of the Declaration of Helsinki and was approved by the local ethics committee. The participants were included in the study after oral and written informed consent had been given.

**Study procedure**

The study was performed as an open parallel-group comparison between women with PRP and healthy females during the winter months of February 1994 and 1995 and the summer month of August 1994. The mean outdoor temperatures during these months were -8.7°C, +2.1°C and +16.6°C respectively.

The participants were instructed not to drink caffeine-containing beverages or to use tobacco 12 h before the investigation. The participants, who were in supine position in a temperature-controlled room (+21°C), had an intravenous cannula inserted into the antecubital vein 30 min before the start of the procedure.

Two blood samples, for the purpose of measuring venous cGMP, were drawn from the antecubital vein. The first blood sample was drawn after 30 min of rest at room temperature. Thereafter, the participants were covered from chin to feet with a water-chilled blanket at a temperature of +13°C for 40 min. The second blood sample was drawn just before the whole-body cooling was terminated.

**Determination of venous cGMP**

Venous blood (10 ml) was drawn into Vacutainer tubes containing EDTA (Becton, Dickinson, Rutherford, NJ, U.S.A.) at a final concentration of 1 mg/ml. Within 15 min after sampling, blood samples were centrifuged at 2000 g for 10 min at 5°C. After the centrifugation, plasma was withdrawn. Plasma proteins were precipitated with 10% (w/v) trichloroacetic acid (1:1) and samples were centrifuged at 3200 g for 15 min at 5°C. The supernatants were collected, frozen and stored at -80°C. Samples were extracted with 4 x 3 ml of water-saturated diethyl ester, as previously described [15]. The aqueous phase was recovered and lyophilized, and the residue was dissolved in 600 µl of 150 mmol/l

<table>
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<th>Time of measurement</th>
<th>n</th>
<th>Age in years (range)</th>
<th>Numbers of smokers</th>
<th>Number of post-menopausal women</th>
<th>Blood pressure (mmHg)</th>
<th>Duration of symptoms in years (range)</th>
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<td>February 1994</td>
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sodium acetate buffer, pH 6.2. The cyclic nucleotide was measured by RIA [10]. The isotope used in this assay was guanosine 3',5',-cyclic-phosphoric acid, [125I]-labelled 2'-O-succinyl (iodosine methyl ester) (New England Nuclear, Boston, MA, U.S.A.).

Statistical analysis

Student's t-test for paired and unpaired observations was used to evaluate the differences in age, blood pressure and venous cGMP between the two study groups. We used two-tailed tests to analyse the differences in venous cGMP between the study groups at baseline. Otherwise the one-tailed test was used. Chi2 tests were used to compare the difference in the number of post-menopausal and smoking women in the study groups. All data in the Tables are expressed as mean values and the 95% confidence interval (CI). A value of P<0.05 was regarded as significant.

RESULTS

Baseline levels of venous cGMP

The basal levels of venous cGMP in women with PRP were significantly lower during the winter months of February 1994 and 1995 compared with the values in August 1994; (A 1.18 pmol/ml, 95% CI from 0.62 to 1.74, P<0.01 and Δ 0.91 pmol/ml, 95% CI from 0.35 to 1.47, P<0.01 respectively). However, the basal levels of cGMP did not show any significant seasonal variation in the healthy controls. (see Table 2)

Venous cGMP and whole-body cooling

There were no significant changes in venous cGMP after whole-body cooling in the PRP group during the winter months of February 1994 and 1995. However, whole-body cooling in the summer month of August resulted in a significant increase in venous cGMP (Δ 0.56 pmol/ml, 95% CI from 0.16 to 0.96, P<0.01). In contrast, the healthy women responded with significant increases in venous cGMP on all three test occasions: February 1994 (Δ 0.43 pmol/ml, 95% CI from 0.02 to 0.85; P<0.05); August 1994 (Δ 0.70 pmol/ml, 95% CI from 0.13 to 1.27; P<0.05); and February 1995 (Δ 0.46 pmol/ml, 95% CI from 0.10 to 0.75; P<0.01). (See Table 2 and Fig. 1).

Subgroups analysis

There were five, or alternatively six, post-menopausal women in the PRP group and only one or two in the control group during the different study occasions. The post-menopausal women in the PRP group showed inconsistent results measured during the two winter months. In February 1994 venous cGMP increased significantly after whole-body cooling (n = 5), Δ 0.96 pmol/ml P<0.05, whereas in February 1995 no significant changes were found.

In the control group, there were only a few post-menopausal women on each test occasion (one, one and two in February 1994, August 1994 and February 1995 respectively).

DISCUSSION

The main finding in the present study was the presence of a seasonal variation in venous cGMP response after whole-body cooling in women with PRP. The levels of venous cGMP in women with PRP increased significantly after cold exposure performed during the summer month of August, whereas a similar reaction was not observed during the two winter months. In contrast, healthy women showed a significant increase in venous cGMP levels.
Fig. 1. Venous cGMP levels before and after 40 min of whole-body cooling measured on three different test occasions in the women with PRP and healthy controls.

Another finding was the significant increase in basal levels of venous cGMP during the summer in women with PRP, whereas this seasonal variation was not observed in the control group.

To eliminate any measurement bias, all samples were analysed by one technician, who did not know to which group the participants belonged. The fact that the majority of the participants took part in all three studies, makes it improbable that the seasonal variation in venous cGMP could be the result of the addition of new subjects.

Such factors as smoking and endogenous oestrogen have a known effect on endothelial function in women [16, 17]. In the present study, the number of smokers did not differ between the two groups. However, subgroup analyses of smokers and non-smokers were difficult to interpret because of the small number of participants in each of the subgroups (type II error). In the Raynaud group, the number of post-menopausal women was higher than in the control group. However, the over representation of post-menopausal women in the PRP groups could not explain the seasonal difference of cGMP between the two groups as the relation between pre- and post-menopausal females was constant on all three test occasions. Furthermore, the lack of increase in venous cGMP after cold exposure in the Raynaud group observed in February 1994 was confirmed on the February 1995 test occasion.

It has previously been suggested that the lack of venous cGMP response on prolonged whole-body cooling during the winter season in women with PRP is caused by an impairment in the L-arginine–NO–cGMP pathway [9]. However, our study showed that during the summer, women with PRP were fully capable of responding with an increase in venous cGMP after whole-body cooling. Gold et al. [18] reported that the basal level of cGMP was influenced by the basal level of L-arginine; i.e. a drop in the level of L-arginine in endothelium-intact arterial rings was accompanied by a marked decline in the tissue levels of cGMP [18]. Therefore, differences in basal levels of venous cGMP and the response on whole-body cooling in summer and winter periods in PRP women may be dependent on the availability of L-arginine and on a diminished capacity in the regulation of constitutive nitric oxide synthase [19].

Even though seasonal variation of clinical symptoms is a well documented fact in PRP, there is only one published report regarding seasonal variation of pathophysiological factors in patients with PRP [11]. That report concerns erythrocyte magnesium, with low values during winter and high values during summer months in women with PRP.

Increased blood viscosity has been suggested as one of the pathophysiological factors underlying Raynaud's phenomenon [20]. Stout and Crawford [21] found seasonal variation of fibrinogen concentration, with higher values during the winter than in the summer, in a group of elderly people without any signs of PRP [21]. Increased sympathetic tone in blood vessels has also been suggested as an aetiological factor in Raynaud's phenomenon [22]. Seasonal variation of sympathetic tone has, however, only been studied in a group of middle-aged people without any signs of Raynaud's phenomenon [23]. As a consequence of the present findings, we suggest that studies concerning pathophysiological factors underlying Raynaud's phenomenon should be performed during both winter and summer seasons of the year.

Conclusion

A seasonal variation in venous cGMP response after whole-body cooling was only observed in women with PRP. Healthy women responded to cold exposure with an increase in venous cGMP both in summer and winter, whereas females with PRP showed an increase only in the summer season. Whether our finding of seasonal variation in the ability to increase venous cGMP in response to whole-body cooling in women with PRP is a primary or a secondary phenomenon, is, however, unclear with regard to the multifactorial and complex mechanism behind vasoconstriction and vasodilatation. We will avoid speculation on how further studies will be performed.

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Seasonal variations in cyclic GMP in Raynaud's phenomenon, seasonal variation

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