Postmenopausal women with vasomotor symptoms have increased urinary excretion of calcitonin gene-related peptide

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Abstract

Objectives: To establish whether 24 h urinary excretion of the potent vasodilator calcitonin gene-related peptide (CGRP) was higher in postmenopausal women with vasomotor symptoms compared to the level in women without symptoms. We also wanted to establish whether urinary excretion of CGRP changed during the menstrual cycle in women of fertile age. Material and methods: Thirteen postmenopausal women with and 13 women without vasomotor symptoms were included. Urine was collected over 24 h and CGRP excretion was measured utilizing radio-immuno assay technique. Twenty-four hour CGRP excretion was also measured in ten fertile women with regular cycles in early follicular, preovulatory and midluteal phase. Results: Twenty-four hour urinary excretion of CGRP was significantly higher in women with vasomotor symptoms compared to non-flushing women (median 7.16 vs 5.15 pmol/24h; P = 0.028). CGRP concentrations were stable throughout the ovulatory cycles. Conclusion: The 24 h urinary excretion of CGRP is higher in women with vasomotor symptoms than in women without these symptoms. CGRP may be the mediator of vasodilator signals originating from the thermoregulatory center. © 1998 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Calcitonin gene-related peptide; Hot flushes; Postmenopausal women; Vasomotor symptoms

1. Introduction

The majority of peri- and postmenopausal women suffer from vasomotor symptoms like hot flushes and episodic sweating, often with decreased well-being, sleep disturbances, with nega-
tive effect on quality of life [1,2]. During the flushes it is possible to objectively register increased pulse rate, skin-temperature, skin-moisture, peripheral vasodilatation and decreased central temperature [3,4]. There is a temporal correlation between the hot flushes and elevations of serum LH in turn caused by a GnRH-pulse [5]. These phenomena are probably elicited by a sudden resetting of the thermoregulatory center [6,7] and by an increase in GnRH pulse rate or amplitude. Thermoregulation and GnRH neurons are both located in the hypothalamus and are also both affected by \( \beta \)-endorphin activity [8,9]. Decreasing estrogen production correlates with low levels of \( \beta \)-endorphin in the hypophyseal portal blood in the female pigtailed monkey [10]. Furthermore, the concentration of \( \beta \)-endorphin was elevated by estrogen treatment [10]. Indirect evidence also suggests decreasing central \( \beta \)-endorphin activity in postmenopausal women, and increasing activity during estrogen treatment [11]. Although this is still speculative, the increased opioid secretion, induced by estrogen treatment [11], may explain some of the beneficial effects of estrogens on vasomotor symptoms.

It has been shown that endogenous opioids modulate the release of the potent vasodilator calcitonin gene-related peptide (CGRP) at the spinal cord level [12]. CGRP is a 37-amino acid peptide widely present in the brain and in the sensory as well as the parasympathetic nervous system [13,14]. It is not only a highly potent endothelium-dependent vasodilator of systemic blood vessels [14,15] but also enhances cholinergic sweating [16] and may on these grounds be involved in the mechanisms behind flushing. We have previously observed that the 24 h urine excretion of CGRP was higher in a group of postmenopausal women with hot flushes before therapy than after 8 weeks of successful treatment with acupuncture [17]. CGRP may thus be a mediator of the efferent signals from the thermoregulatory center to the periphery in order to achieve vasodilation in the hot flushes.

It is not known whether CGRP excretion is stable or varies with the changing sex steroid concentrations throughout the menstrual cycle. Furthermore, there are few data on whether CGRP differs between women with and without vasomotor symptoms or whether it changes in postmenopausal women before and after estrogen therapy.

The objective of this study was to establish whether 24 h urine excretion of CGRP was higher in postmenopausal women who had vasomotor symptoms as compared to women without symptoms. We also wanted to establish whether the urinary excretion of CGRP changed during the menstrual cycle in fertile women.

2. Material and methods

The study was performed on two subgroups of women. The first involved 26 healthy postmenopausal women. None of these women used any medication and they had no signs of any metabolic or endocrine disease. Thirteen of the women were consecutively recruited from the outpatient clinic where they presented with vasomotor symptoms. Their median age was 54 years, time since menopause was at least 6 months and none had used hormone replacement therapy for at least 6 months (Table 1).

The other 13 women in this group were without any vasomotor symptoms. They had answered a population-based postal questionnaire on vasomotor symptoms and were invited to the study by means of a letter since they had reported no vasomotor symptoms. Their median age was 57 years, time since menopause was at least 6 months (Table 1). All women were asked to collect urine over 24 h for measurement of CGRP excretion. Furthermore blood samples were drawn for measurements of serum concentrations of FSH and estradiol.

The second part of the study comprised ten young healthy women, who all reported regular menstrual cycles. During two complete cycles they measured basal body temperature and collected urine over 24 h for measurement of CGRP excretion during early follicular phase (cycle day 2–4), preovulatory phase (day 12–14) and midluteal phase (cycle day 19–23). A blood sample was taken at the midluteal visit for measurement of progesterone concentrations. Two women only
Table 1
Data on 13 women without vasomotor symptoms and 13 women with moderate to severe vasomotor symptoms

<table>
<thead>
<tr>
<th></th>
<th>Women with vasomotor symptoms</th>
<th>Women without vasomotor symptoms</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median 25–75th perc</td>
<td>Median 25–75th perc</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>54</td>
<td>57</td>
<td>NS</td>
</tr>
<tr>
<td>Months since menopause</td>
<td>24</td>
<td>60</td>
<td>0.033</td>
</tr>
<tr>
<td>Flashes/week</td>
<td>58</td>
<td>0</td>
<td>0.002</td>
</tr>
<tr>
<td>Kupperman's Index</td>
<td>25</td>
<td>2</td>
<td>0.005</td>
</tr>
<tr>
<td>FSH (U/l)</td>
<td>47</td>
<td>62</td>
<td>NS</td>
</tr>
<tr>
<td>Estradiol (pmol/l)</td>
<td>51</td>
<td>40</td>
<td>NS</td>
</tr>
<tr>
<td>CGRP (pmol/l)</td>
<td>5.42</td>
<td>3.22</td>
<td>0.002</td>
</tr>
<tr>
<td>CGRP (pmol/24h)</td>
<td>7.16</td>
<td>5.15</td>
<td>0.028</td>
</tr>
<tr>
<td>24 h Urine volume (ml)</td>
<td>1334</td>
<td>1527</td>
<td>NS</td>
</tr>
</tbody>
</table>

P-values according to Mann–Whitney U test.

collected urine during one cycle each, one woman appeared to be irregular during the time of the study and hence samples from both her cycles were omitted. Samples from one of the cycles from yet another woman could not be used, because they were destroyed during transport. In all, urine was therefore collected and analyzed from 15 ovulatory cycles. Women were asked not to exercise during the period they collected urine, to avoid possible influence from exercise and temperature variation on CGRP excretion.

Written and oral consent were obtained from all women.

Monitoring: All postmenopausal women registered the number of flushes daily in a special logbook during two weeks before urine was collected over 24 h. They registered the number of attacks per day and night, respectively, each according to their own estimate. Climacteric symptoms were evaluated using a modified form of the Kupperman Index [18].

Analysis of urinary peptides: The 24 h urine collected was rapidly cooled and stored at −70°C until the peptide analysis was performed. Samples were extracted using a reverse-phase C18 cartridge (Sep Pak, Waters) and analyzed for calcitonin gene-related peptide-like immunoreactivity (CGRP-LI) using a competitive radioimmunoassay [19].

Calcitonin gene-related peptide-like immunoreactivity (CGRP-LI) was analyzed using antiserum CGPR8 raised against conjugated rat CGRP. HPLC-purified (125) I-histadyl rat CGRP was used as radioligand and rat CGRP as standard. The cross-reactivity of the assay to SP, adrenomedullin, neurokinin A, neurokinin B, neuropeptide K, gastrin, islet amyloid polypeptide, neurotensin, bombesin, neuropeptide Y and calcitonin was less than 0.01%. Cross-reactivity towards human CGRP α and β was 93 and 24%, respectively and towards rat CGRP α and β 100 and 120%, respectively. Intra and interassay coefficients of variation were 8 and 14%, respectively.

Ethics: The study was approved by the local ethical committee at the University.

Data handling and statistics: The data was coded and remained so when analyzed using Macintosh Statview 4.1 (Abacus Concepts). Results are given as medians and 25 to 75th percentiles.

The nonparametrical Mann–Whitney U test was used when analyzing differences between groups.

3. Results

Postmenopausal flushing women were slightly albeit non-significantly younger and somewhat closer to menopause than women without flushes (Table 1). Postmenopausal hormonal status was confirmed by low serum concentrations of estradiol and high FSH concentrations (Table 1).
Both CGRP concentration and total excretion in 24 h urine was significantly higher ($P = 0.002$ and $P = 0.028$, respectively) in the women with hot flushes than in the postmenopausal women who did not suffer from vasomotor symptoms (Table 1).

In 15 cycles of the fertile women ovulatory cycles were verified with a biphasic temperature and high midluteal serum progesterone concentrations. CGRP excretion in 24 h urine was analyzed in urine collected during these 15 cycles. Median (25–75th percentiles) 24 h urinary excretion of CGRP was 3.9 pmol (2.8–5.6) in early follicular phase, 4.4 pmol (2.7–5.4) in preovulatory phase and 3.7 pmol (3.1–5.3) in midluteal phase. Thus, CGRP excretion did not change significantly throughout the menstrual cycle and the median CGRP excretion out of the 45 samples (15 from each three phases of the menstrual cycle) was 4.0 pmol:24 h (2.9–5.4). Although CGRP excretion in 24 h urine was lower in the fertile group compared to the postmenopausal groups, we have not analysed this statistically as these laboratory analyses were performed in different assays.

### 4. Discussion

Since CGRP has a significant circadian rhythm [20], and also, according to the profiles published by Valentini et al. [21], seems to have a short half-life in plasma, we found it reasonable to analyse the 24 h urinary excretion of the peptide. Thereby we avoided the risk of measuring randomly high or low blood levels. Not all CGRP produced may be detected in urine because urinary excretion may depend on age and renal function. All the women in our study were, however, healthy and without signs of renal dysfunction. Furthermore, there may be some spontaneous degradation of the peptide during for example storage in the bladder, but there are today no available data on these methodological problems.

With these methodological drawbacks, we found that urinary excretion of CGRP was significantly higher in the women with hot flushes compared to those without vasomotor symptom, but we do not know whether our finding is a cause or an effect. Low CGRP urinary excretion in women without vasomotor symptoms may be either the reason why they do not flush or be caused by the fact that their central thermoregulation was more stable and did not initiate a decrease of the central body temperature.

It could be argued that we did not objectively measure flushes in our study groups. It has been clearly shown, however, that there is a very high, almost total, correlation between objectively measured and subjectively registered hot flushes in postmenopausal women [22].

Valentini et al. [21] collected blood samples at 20 min intervals and every minute at the occurrence of hot flushes and found a two to four-fold increase in CGRP concentrations during the flush in 12 climacteric women. This supports the hypothesis that CGRP is involved in peripheral thermoregulation by means of regulating vasodilation. In the same study, however, they made two other observations which were not in line with the other findings. They found that plasma CGRP concentrations were higher in 11 healthy fertile women during follicular phase than in post-menopausal, flushing women. Furthermore, plasma CGRP concentrations were higher after 3 months of hormone replacement therapy (HRT) than before therapy. The authors concluded that low CGRP concentrations after menopause seem to be related to estrogen deficiency but gave no explanation to their observations about CGRP levels before and after HRT. An explanation could be that they compared CGRP concentrations in single blood samples drawn before and after HRT, although plasma CGRP concentrations fluctuate rapidly in relation to hot flushes. There is, thus, a risk that the concentration of CGRP in a single blood sample is affected by the situation as such or by an ongoing flush.

To decrease the risk of measuring random fluctuations in CGRP we measured CGRP in urine collected over 24 h. With this method we found that women with vasomotor symptoms had higher urinary excretion of CGRP than non-flushing women. In line with our findings, Chen et al. [23] found that serum concentrations of CGRP were higher in six postmenopausal women with at
least ten hot flushes per day than in 18 women without flushes. Furthermore, the women with flushes had higher serum concentrations of CGRP during than after the flush [23].

In an effort to explain the different and somewhat contradictory findings about CGRP in postmenopausal women [17, 21, 23], we may speculate that CGRP production in peripheral nerves is decreased after menopause under low estrogenic influence. This would lead to a tendency to vasoconstriction and decreased heat loss. Decreased heat loss would in turn slowly increase central temperature, and intermittently, the thermoregulatory centre would try to restore temperature, by vasodilation and sweating. This is in line with data from Freedman and Woodward, who found that elevations in core body temperature precede most menopausal hot flushes [24]. The vasodilation seen during the flush may involve and even be caused by a sudden CGRP release, which may be measured as increased CGRP in plasma [21, 23]. Intermittent CGRP release may lead to a higher 24 h urinary excretion of CGRP in flushing than in nonflushing women, as observed in the present study and in a previous study on women treated with acupuncture for vasomotor symptoms [17]. On the other hand, the CGRP concentrations in single blood samples drawn between flushes may even be lower than the more stable concentrations in nonflushing postmenopausal women or women of fertile age.

According to this explanation, when a postmenopausal woman receives HRT her thermoregulation and also her CGRP production becomes more stable. After HRT has been given to a woman with flushes, the CGRP concentrations in serum may on average be the same or even higher, as in the study by Valentini et al. [21], than was found between the flushes before HRT was initiated.

It should be emphasized that this is merely an attempt to explain and put together the different observations, which have so far been reported on CGRP and vasomotor symptoms. Actually, we still do not know what CGRP affects in postmenopausal women with and without vasomotor symptoms. There are, however, data about effects of CGRP administered to healthy male volunteers. Intravenous administration of a small dose of CGRP during 3 min produced an increase in cutaneous blood flow during 5–10 min, measured by Laser Doppler flowmetry, whereas larger doses increased the blood flow for 30–90 min [25]. The systemic infusion also induced a flush in the face, neck, upper arm and upper trunk.

CGRP excretion was unaffected by hormone fluctuations during the menstrual cycle, suggesting that sex steroids on their own do not affect CGRP excretion as long as thermoregulation is unaffected and the woman does not have flushes. A small number of premenopausal women, however, report premenstrual vasomotor symptoms [26, 27] and perhaps cyclic changes in CGRP excretion could be found in these women. Estrogen treatment has been shown to affect CGRP content in the rat pituitary, and CGRP is synthesised by preoptic neurones containing estrogen receptors, showing that sex steroids may also have direct effects on the synthesis and secretion of the peptide in the central nervous system [28, 29].

In conclusion the peptide CGRP appears to be involved in the mechanisms behind hot flushes in postmenopausal women. Since the exact mechanisms are still not understood the relation between CGRP and hot flushes should be subjected to further studies.

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