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Hot flushes in healthy aging men differ from those in men with prostate cancer and in menopausal women.

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Abstract:

Calcitonin Gene-Related Peptide (CGRP) seems to be involved in hot flushes in women and in castrated men. Therefore, we studied whether the plasma concentrations of CGRP changed during flushes in a group of healthy aging men. Twelve men (49-71 years) with no history of current or former prostate cancer or hormonal treatment reporting ≥ 20 flushes/week were investigated. Blood samples were drawn during and between flushes for analysis of CGRP and also androgen concentrations, i.e. Testosterone and Bioavailable Testosterone were analysed. Skin temperature and skin conductance were monitored.

35 flushes were reported by 10 men. The plasma concentrations of CGRP did not increase during flushes. No significant change in skin temperature or conductance was found.

CGRP is probably not involved in the mechanisms of flushes in healthy aging men.

Therefore, flushes in aging healthy men seem to be different from flushes in men and women deprived of sex-steroids where CGRP increases during flushes.

Introduction

Hot flushes are classic menopausal symptoms in women [1, 2] and also reported by 43-77 % of men after castration therapy [3,4]. Hot flushes occur in men with testicular insufficiency and in “normal aging men”, but to a lesser extent [5,6]. Nearly 100% of reported flushes in women and men after castration therapy are objectively confirmed by increased blood-flow, skin-conductance and/or skin-temperature [7-10].

Endogenous opioids modulate the release of the potent endothelium-dependent vasodilator calcitonin gene-related peptide (CGRP) at the spinal-cord level [11,12]. When CGRP is administrated intravenously to healthy male volunteers it produces symptoms very similar to hot flushes, with a dose-dependent increase in cutaneous blood flow [13]. CGRP increases in plasma during hot flushes in menopausal women [14-16] and according to one study, in men with flushes who had been castrated due to prostate cancer [10]. No relation has been reported between flushes and CGRP in non-castrated aging men, but flushes in these men have not been given much attention. We therefore investigated whether CGRP also is involved in hot flushes in normal aging healthy men, monitoring both skin-temperature and skin-conductance simultaneously.

Methods

Participants

Twelve men were recruited by advertisement in the local press in Linköping, Sweden. Men who suffered from daily hot flushes ($> 20/\text{week}$) that had appeared after the age of 45, who had not had sexual hormone treatment or a history of prostate cancer were included. Exclusion criteria were uncontrolled hypertension, severe angina pectoris, recent myocardial infarction, anaemia ($\text{Hb} < 120 \text{ g/L}$) as well as use of psychoactive drugs the week before the investigation. Long-term antidepressant medication was allowed if the treatment had been on-going for a long time and if the start of hot flushes did not coincide with the start of or changes in the dose of the treatment.

Procedure

The men attended the outpatient clinic at the Division of Obstetrics and Gynaecology, University Hospital of Linköping, for one whole day, during which time they were not allowed to smoke or drink coffee, tea or alcohol. Venous blood-samples were taken for analysis of Testosterone (T), Follitropin (FSH), Lutropin (LH), Sex Hormone Binding Globulin (SHBG), albumin and capillary haemoglobin (Hb) and Bio-available Testosterone (BT) was calculated [20].

At four different occasions during the day, after at least 30 min of rest in supine position without hot flushes, blood-samples were taken for measurement of basal plasma CGRP-concentrations (CGRP basal). The continuous monitoring of skin-conductance and skin-temperature was interrupted only in connection with toilet visits.

When the man signalled a flush coming, blood samples for analyses of CGRP were taken (CGRP flush) after 2, 3 and 4 minutes.

Laboratory measurements

All analyses were carried out at the Department of Clinical Chemistry, University Hospital, Linköping, Sweden, accredited according to ISO 17025.

Albumin was analysed using nephelometry (Advia 1650, Bayer Sweden AB, Sweden). T, Albumin, FSH, LH and SHBG were measured using immunochemical techniques based on time-resolved Europium fluorescence detection (AutoDelfia 1235, Wallac, Turku, Finland). BT was calculated on basis of T, Albumin, and SHBG according to an algorithm previously described [17].

Analysis of CGRP

Cold 6 mL tubes were filled with venous blood. 300 μ L aprotinin was added and the tubes were incubated on ice for twenty minutes, centrifuged at 3,500 x G for 10 minutes at -4° C. Thereafter plasma was taken off and stored at -70°C until analysis.

Samples were extracted and concentrated five times using a reverse-phase C18 cartridge (Sep Pak, Waters) and analysed for CGRP-like immunoreactivity (CGRP-LI), using competitive radioimmunoassays [18]. CGRP-LI was analysed using antiserum CGRPR8 raised against conjugated rat CGRP. HPLC-purified ¹²⁵I-histidyl rat CGRP was used as radioligand and human CGRP as calibrator. The cross-reactivity of the assay to SP, neurokinin A, neurokinin B, neuropeptide K, gastrin, neurotensin, bombesin, islet amyloid polypeptide, adrenomedullin, neuropeptide Y and calcitonin was less than 0.01%. Cross-reactivity toward human CGRP α and β was 93% and 24%, respectively, and toward rat CGRP α and β 100% and 120%, respectively. Intra- and interassay coefficients of variation were 8 and 14% respectively. The lower limit of detection in the original samples was 0.4 pmol/L for CGRP.

Skin-temperature

The skin-temperature was recorded from the dorsal surface of the midphalanx of the third finger of one hand using a MP100 system with a SKT100B amplifier from the BioPac Systems Inc., U.S.A.

Skin-conductance

The skin-conductance (SC) was recorded from the pre-sternal area using Ag-AgCl electrodes with self-adhesive electrolyte layer offering a distinct active electrode area of 2.5 cm². The two electrodes were attached approximately at the left frontal dermatome T2 with a distance of 5 cm between electrodes. A constant voltage (0.5 V) was applied across the electrodes according to standard procedure [19]. A GSR100B amplifier of the MP100 system, BioPac Systems Inc., U.S.A, accomplished the measurements.

Visual analogue scale rating of flush-intensity

The flush-intensity was estimated on a visual analogue scale (VAS) from 1 to 10, 1 being no complaints at all and 10 being the most intense hot flush he could imagine.

Ethics

Written as well as oral information was given. The local ethical committee at the University of Linköping approved the study.

Statistical analysis

Data were analysed using STATA (9.2, Collage Station, Tx, USA).

Because of the limited amount of material non-parametric tests were applied as well as parametric tests when appropriate. The Student's *t*-test and Wilcoxon Matched-pairs Signed-ranks test were used when analysing pair-wise differences in peptide concentrations and skin conductance between non-flush and flush periods and also between men with higher BT-, T-, T/SHBG-levels and VAS-scores compared to lower levels and scores respectively. *Chi*² test was used to compare the number of reported flushes that were detected by SC measurements compared to the results from an earlier study by us [10]. The rejection limit for the null-hypothesis was set to $\alpha = 0.05$. All statistical tests were two-sided.

Results

Clinical data and hormonal status are presented in Table I. Psychoactive drugs had been used by two men in unchanged dose during a period lasting many years including the time at the start of their hot flashes. Five men used hypertensive medication.

Ten men reported a total of 35 hot flushes (median 4; range 1-5 per man). All flushes were analysed via blood-samples. The means of CGRP basal and CGRP flush concentrations did not differ significantly and the VAS score of the flushes was low.

We divided the patients into two groups (Group I and II) on the basis of the median levels of BT, T and T/SHBG. There were no significant differences between the numbers of flushes, CGRP basal, CGRP flush or VAS in the two groups and not according to the two groups of low and high (Group I and II) concentrations of BT, T and T/SHBG, respectively (Table II).

Skin-conductance and skin-temperature

In total 38 flushes were either reported and/or detected by SC. In one man, no flushes were reported, but the SC measurements detected three flushes during the day. Due to disk-crashes skin conductance recordings and skin temperature measurements were lost for one man for an entire day, and partially for five men. We thereby lost recordings from 11 reported flushes, with the result that a total of 24 reported flushes constitutes the material for the study in this respect. Of these 24 flushes, only 9 were both reported and detected by SC (Table III).

The magnitudes of the increase of SC were calculated for the nine recorded flushes. The mean conductance magnitude of all reported hot flushes was calculated for each subject. The mean magnitudes (magnitude of all mean of the mean magnitudes within subjects) was 5.2 μ S which constituted an increase of 129% from immediately before the flushes ($p=0.0017$). Fifteen of the reported flushes were not objectively confirmed at all on SC recordings. CGRP flush did not differ during and between these objectively and subjectively reported flushes.

In four men, a small continuous basal sweat-activity was recorded by SC measurements throughout the day. All of these men reported hot flushes during the day that were not objectively confirmed. The skin-temperature recordings were very variable over the day in all men. Changes in SC and skin-temperature during recorded flushes are shown in Table III.

Discussion

CGRP did not increase consistently in plasma during flushes in healthy aging men in this study and the majority of hot flushes were not objectively detectable from changes in skin temperature or skin conductance. This in contrast to the results from menopausal women [14-16] and according to one study of men castrated due to prostate cancer [10].

We postulated that the mechanism causing flushes in elderly men without castration therapy may be similar to the mechanisms operating in menopausal women and castrated men. However, only 9 of 24 reported and recorded flushes were objectively detected by the skin-conductance measurements in ordinary elderly men. This is in great contrast to the study on castrated men where all flushes were both reported and objectively detected by increased skin-conductance; in addition, skin-conductance increased by 394 % [10] during detectable flushes in castrated men, compared to 129% in the current study.

Flushes in menopausal women are preceded by small increases in core-body temperature [20,21]. This relationship has not been investigated or reported in healthy aging men with flushes as far as we know. Recently, Hanish et al demonstrated increases in core body temperature before flushes in a prostate cancer patient [22]. Since we were not able to detect changes in peripheral temperature or skin conductance during subjectively reported flushes in this study, it is not likely that an increase in core body temperature preceded the flushes in this study group.

T`Sjoen et al [23] investigated 161 healthy men 74-89 years old who completed the "Aging Males Symptoms rating scale" (AMS), developed by Heinemann et al 1999 to predict low androgen concentrations [24,25]. The results showed mild, moderate, or severe flushes in 41.1%, 40.5% and 7.1%, respectively. However, the results of the AMS could not be significantly correlated with T- and BT-concentrations, as in many other recent studies [23,25,26]. Specific treatment for flushes in aging men is therefore difficult to advice. Estrogen-therapy alleviates hot flushes in women, and testosterone given to men treated by castration due to prostate cancer,

(although contraindicated), alleviates flashes. In men with androgen ablation therapy alternative treatments include therapies such as acupuncture, diethylstilbestrol, gabapentin, venlafaxine, medroxyprogesterone acetate and cyproteroneacetate [27-30]. To our knowledge, no controlled treatment trials have been performed in healthy aging men with flushes.

It can be argued that we had too low power in our study to prove our hypothesis. However, we dimensioned the study based on our former studies on women and men deprived of sex-steroids [10,16]. Unfortunately, we also had problems with the SC recordings due to computer disc crashes.

In conclusion, we were not able to identify increasing plasma-CGRP concentrations in the blood during flushes in non-castrated men and the majority of subjectively reported flushes were not objectively confirmed by increases in skin-conductance or skin-temperature. These findings may indicate that flushes in aging healthy men differ from hot flushes in men and women deprived of sex-steroids and are to be explained, perhaps, by a more multi-factorial origin.

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Declaration of Interest statement:

The authors report no declarations of interest.

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Table I: Clinical data and hormonal status for the patients included

Pat no	Age (Years)	BMI kg/m ²	T (nmol/L)	BT (nmol/L)	LH (U/L)	FSH (U/L)	SHBG (nmol/L)	Albumin (g/L)	Hb (g/L)
1	56	31,1	16	--	10.7	2	25	--	135
2	56	30.1	15	--	2.1	6.3	37	--	152
3	58	29.9	21	8.4	3.3	3.5	49	39	161
4	55	28.0	17	8.3	3.3	6.2	38	45	164
5	62	37.1	10	4.9	2.8	3.2	33	43	153
6	71	27.4	16	6.4	5.4	15	44	37	164
7	55	32.4	6.6	5.2	5.3	3.9	9.5	49	169
8	57	30.9	19	8.1	3.5	8.5	49	46	162
9	56	38.2	7.2	3.3	3.2	1.5	37	44	138
10	49	27.5	8.5	5.5	3.2	4.1	18	43	149
11	58	--	5.1	3.7	2.2	2.3	11	39	146
12	54	23.0	24	9	2.5	3.6	59	42	167
Mean	57.3±5.3	30.5±4.3	13.7±6.2	6.3±2.1	4.0±2.4	5.0±3.73	34.1±15.6	42.7±3.6	155±11.3
±SD									
"--" data missing									

Table II:

Mean number of hot flushes, concentrations of CGRP-basal and CGRP-flush and intensity of flushes according to VAS in groups according to BT, T and T/SHBG-levels. 95%CI within

brackets.

	Group I	Group II	
BT	<5.95 nmol/L	≥5.95 nmol/L	
	n = 4	n = 4	p
Number of hot flushes	4.0 (2.70-5.30)	3.3 (1.73-4.77)	ns
CGRP basal	3.1 (1.55-4.70)	2.6 (1.90-3.34)	ns
CGRP flush	3.9 (0.85-6.96)	2.48 (1.88-3.08)	ns
VAS	3.5 (2.29-4.81)	4.1 (1.39-6.81)	ns
T	<15.5 nmol/L	≥15.5 nmol/L.	
	n = 5	n = 5	p
Number of hot flushes	4.2 (3.16-5.24)	2.8 (1.18-4.42)	ns
CGRP basal	2.7 (0.96-4.34)	2.4 (1.51-3.22)	ns
CGRP flush	3.3 (0.64-5.96)	2.3 (1.79-2.90)	ns
VAS	3.3 (2.20-4.40)	3.7 (1.51-5.85)	ns
T/SHBG	<0.415	≥0.415	
	n = 4	n = 6	p
Number of hot flushes	4.3 (3.45-5.05)	3.0 (1.52-4.48)	ns
CGRP basal	2.6 (0.55-4.71)	2.4 (1.45-3.40)	ns

CGRP flush	2.5 (0.69-4.29)	3.0 (1.12-4.98)	ns
VAS	3.65 (1.07-6.23)	3.4 (2.09-4.68)	ns

Table III: Changes in skin conductance (ΔSC) in micro Siemens (μS) and changes in skin temperature (ΔST) in Celsius degrees for recorded and signalled flushes.

Patients no	Flash no 1		Flash no 2		Flash no 3		Flash no 4		Flash no 5	
	ΔSC	ΔST	ΔSC	ΔST	ΔSC	ΔST	ΔSC	ΔST	ΔSC	ΔST
1	7.058	0.00								
2	7.520	0.0001	10.12	0.0005	10.12	0.0005	-----		-----	
3	0.00	0.00	0.0684	0.0046	-----					
4	2.694	0.0034	0.100	0.033						
5*	8.926*	0.085*	8.5153*	0.085*	5.798*	0.085*				
7	0.34	0.001	2.36	0.0062	1.101	0.0094	2.54	0.0066	-----	
9	-----		-----		0.93	0.0034	0.53	0.0004		
10	-----		7.022	0.0044	6.44	0.0041				
11	0.546	0.0031	0.000	0.00	0.276	0.0028	0.594	0.009		
12	1.185	0.018	0.417	0.0079	-----		-----			

“—” means that the recordings of ΔSC and ΔST is lost

Patient no 6; no flushes reported or recorded

Patient no 8; all recordings lost for ΔSC and ΔST

* ΔSC and ΔST is also given for patient no 5, with no reported flush, but 3 flushes recorded physiologically.