Experimental Stressors Alter Hypertonic Saline-Evoked Masseter Muscle Pain and Autonomic Response

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Aims: *To test in a randomized controlled trial, if hypertonic saline (HS)–evoked pain and autonomic function are modulated by either a cold pressor test (CPT) or mental arithmetic stress induced by a paced auditory serial addition task (PASAT).* ***Methods:*** *Fourteen healthy women participated in three sessions. Pain was induced by two 5% HS infusions (5 minutes each, 30 minutes apart) infused into the masseter muscle. During the second HS infusion, pain was modulated by PASAT, CPT, or control (HS alone). HS-evoked pain intensity was scored on a 0 to 10 numeric rating scale (NRS). Heart rate variability (HRV) and hemodynamic measures were recorded non-invasively (Task Force Monitor). Data were analyzed using repeated measurements ANOVAs and Spearman correlation analysis.* ***Results:*** *HS-evoked pain was significantly and similarly reduced by both PASAT (30.8 ± 27.6%; P < .001) and CPT (35.8 ± 26.6%; P < .001) compared with the control session (9.0 ± 30.5%; P > .05). PASAT and CPT increased the heart rate compared with control (P <.001). CPT reduced measures of vagal activity: Root mean square successive difference, high-frequency (HF) power, and coefficient of HF component variance compared with an internal control, ie, the first HS infusion (P < .05), while PASAT did not alter any of these HRV measures (P > .05).* ***Conclusion:*** *CPT and PASAT reduced HS-evoked masseter muscle pain and altered the autonomic response. The increase in heart rate following CPT and PASAT may be caused by different mechanisms. CPT reduced measures of efferent cardiac vagal (parasympathetic) activity, while the PASAT-induced increase in heart rate, but unchanged HRV, may suggest neurohumoral activation.*
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Key words: autonomic nervous system, cold pressor test, experimental muscle pain, mental stress, trigeminal nociception

Pain and various types of acute stressors activate the autonomic nervous system and cause a series of events; for example, a rise in heart rate and blood pressure, the release of stress hormones, and respiratory changes. This physiological "fight or flight" response is an important defense mechanism in acute situations in which potential or actual tissue damage to the organism exists.[1,2] In contrast, the autonomic nervous system may play a central role for the chronification of pain.[3,4]

Temporomandibular disorders (TMD) are the most prevalent subgroup of orofacial pain conditions. However, the underlying

pathophysiological mechanisms that cause pain in these disorders are not entirely understood.[5,6] An increased urinary level of the catecholaminergic neurotransmitters norepinephrine and epinephrine, released in response to stress, has been demonstrated in patients suffering from chronic myofascial TMD,[7] suggesting that altered sympathetic activity may influence their perception of pain.[3] Sympathetically maintained pain is well known in subgroups of neuropathic pain patients and in patients with complex regional pain syndrome.[8–10]

Pain perception as such is a complex process that depends on various facilitating and inhibiting factors.[11] Pain chronification could be partly due to either an increased facilitation or a decreased endogenous pain inhibition, or both.[12,13] Several different endogenous pain-inhibitory mechanisms have been demonstrated. One mechanism is hypertension-related hypoalgesia, a term that accounts for the inverse relationship between acute pain sensitivity and resting blood pressure.[14–16] Generally, high resting blood pressure is associated with decreased sensitivity to noxious thermal and ischemic stimuli,[14,15] and subjects with increased resting blood pressure have less prevalence of chronic musculoskeletal pain.[17] The physiological mechanism in hypertension-related hypoalgesia is not fully understood. Various possible mechanisms have been suggested (eg, endogenous opioid activity or noradrenergic activity),[16] but several studies have pointed to the interaction between the baroreceptors and pain-regulatory processes in the central nervous system as the underlying mechanism.[13,15,18]

Stress-induced analgesia is another endogenous pain-inhibitory mechanism in which pain-modulatory processes have been shown to inhibit ascending nociceptive information for a short period.[19] Stress-induced analgesia may involve both endogenous opioids and non-opioid mechanisms.[20] Additionally, mechanisms such as conditioned pain modulation, where pain stimuli in one area of the body inhibits pain in another area[21,22]; placebo analgesia, where belief and expected pain reduction results in analgesia[23]; and distraction-induced analgesia, in which cognitive approaches have pain inhibitory effects,[24,25] are other examples of endogenous pain inhibition.

To study the above-mentioned endogenous pain-inhibitory mechanisms in an experimental model mimicking some of the manifestations of TMD, the authors wanted to modulate hypertonic saline (HS)–induced muscle pain by two different stressors: a mental arithmetic task (Paced Auditory Serial Addition Task, PASAT)[26,27] and a cold pressor test (CPT).[21,28] The autonomic involvement was evaluated by heart rate variability (HRV), which is regarded as a measure of autonomic nervous system function and is considered an indirect biomarker of how effectively an organism responds to stress-inducing factors.[29] A recent study in myofascial TMD patients has demonstrated a reduced nocturnal HRV compared with healthy controls.[30] Also, findings from the OPPERA Study[31] have revealed significant differences in autonomic function between TMD patients and healthy subjects when exposed to stressful tasks and during rest.

The aim of the present study was to test, in a randomized controlled trial, if HS–evoked pain and autonomic function are modulated by either a CPT or mental arithmetic stress induced by PASAT. HRV and hemodynamic responses to CPT and PASAT were compared to determine whether these two tests have similar or different effects on autonomic tone. The study specifically tested the following hypotheses: *(1)* acute stress from the CPT or PASAT reduces the intensity of experimentally induced masseter muscle pain compared with control and *(2)* acute stress from CPT and PASAT induces unique patterns of autonomic and cardiovascular responses that differ from each other and from control.

Materials and Methods

Participants

Sixteen healthy women (mean age, 22.9 ± 2.4 years) participated. They were recruited by advertisements at the Aarhus University campus and at the webpage www.forsøgsperson.dk (similar to www.sciencevolunteer.com), and they were compensated for their participation. All volunteers received both written and oral information about the experiment before they signed an informed consent document. The experimental sessions were performed at the Danish Pain Research Center, Aarhus University Hospital, and all data were collected by the same female investigator (KHB). All 16 volunteers completed the study, but data from two subjects were excluded due to technical failures. General exclusion criteria were: TMD according to the Research Diagnostic Criteria for TMD (RDC/TMD)[32]; abnormal electrocardiogram (ECG); inability to read and understand the written information concerning the study; chronic or recurrent headaches or other orofacial pain conditions; general musculoskeletal pain disorders; cardiovascular disease; lung insufficiencies including asthma; previous sympathectomy; diabetes; malignancy; human immunodeficiency virus (HIV); alcoholism or drug abuse; pharmacological treatment affecting the vascular or the autonomic

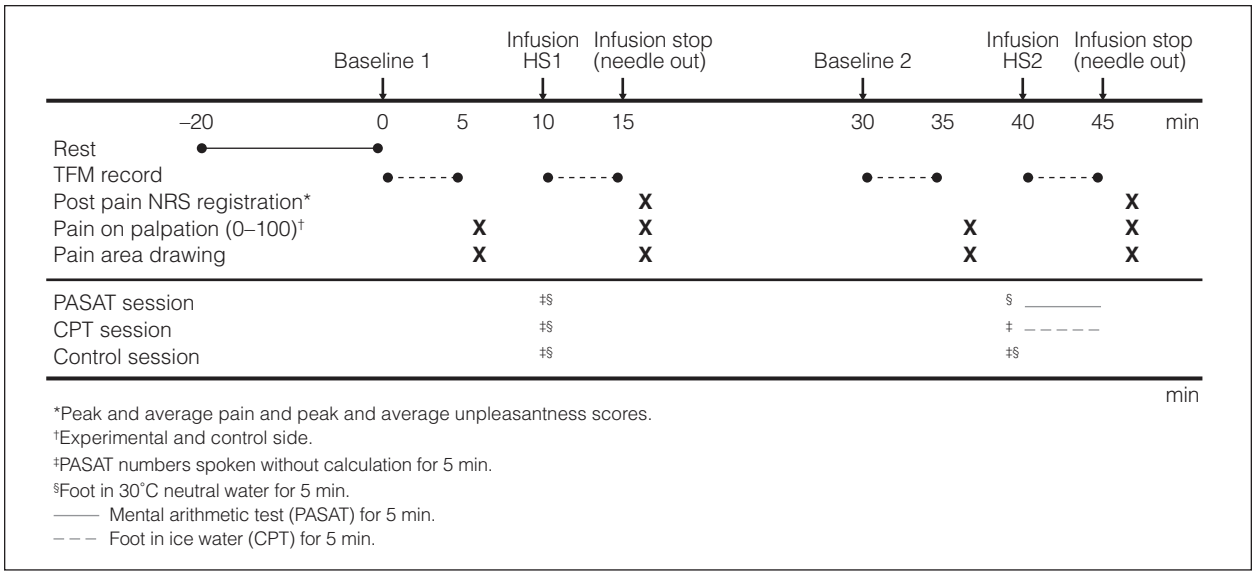

Fig 1 Experimental design. The three sessions (PASAT, CPT, control) were in randomized order.

nervous system; pregnancy, including pregnancy planning and fertility treatment; breastfeeding; and postmenopause.

All volunteers agreed to refrain from intake of coffee, tea, and other caffeinated beverages/foods, as well as alcohol, for the 12-hour period prior to an experimental session. They were obliged to fast for a minimum of 2 hours before participation and to refrain from smoking and excessive physical activity 12 hours before participation. They were not allowed to use any drugs for a minimum of 24 hours before an experimental session. They must not have served as volunteers in other research projects during the month before participation in the present study.

Study Design

The study was performed in accordance with the guidelines of the World Medical Association Declaration of Helsinki and approved by the Central Denmark Region Committees on Biomedical Research Ethics (No. 20080054). It was registered with The Danish Data Protection Agency, Copenhagen, Denmark (No. 2008412790).

During experimental sessions, subjects were positioned comfortably in the supine position in a quiet room with a mean temperature of about 23°C. All sessions were performed in a standardized manner. During experimental recordings (baselines and infusions), the subjects were not allowed to speak unless an emergency situation occurred.

The study was performed as a randomized controlled crossover design (Fig 1). All subjects participated in three experimental sessions (PASAT, CPT, and control) in randomized order with an interval of at least 7 days between sessions. In each session of approximately 90 minutes duration, subjects received two 5-minute painful HS infusions, 30 minutes apart. The first HS infusion (HS1) served as an internal control for variations between sessions[33,34] and as a control infusion within session. The second HS infusion (HS2) was administered during the performance of PASAT, CPT, or control.

Experimental Pain Model

Sterile HS 5% was infused to induce experimental masseter muscle pain. The use of HS is a validated, safe, and widely used model to evoke experimental deep tissue pain, ie, muscle pain.[34–36] HS is a nonspecific, painful stimulus that activates nociceptive masticatory muscle group III and IV afferents.[35,37] Each infusion was administered by the use of B.Braun Perfusor Space syringe pump (B.Braun Melsungen AG) through a disposable syringe and 27-gauge hypodermic needle placed into the deep central segment of the right masseter muscle. B.Braun provided custom-made software for an automatic standardized infusion paradigm according to the requirements of the study design. Initially a bolus of 0.14 mL HS, infusion rate 51.42 mL/h, was infused to evoke pain, followed by a maintenance infusion rate of 6 mL/h, which lasted until 5 minutes of HS infusion was achieved. In total, 0.60 mL HS was administered per infusion. The needle remained inside the muscle tissue for the entire 5-minute duration of

the infusion. A new disposable needle was used for each infusion.

Stressors

Mental Stress Test. During PASAT (+HS2), the subjects were, through headphones, presented with a random sequence of digits from 1 to 9 with a constant interval of 2.4 seconds between each digit for a fixed period of 5 minutes duration.[26] The last two presented digits were continuously added by the subject, and this sum was immediately spoken out loud continuously during the entire 5 minutes. Subjects were requested to concentrate on the task and to score as many correct answers as possible. All scores were recorded, and the percentage of correct answers in total for the 5-minute task was subsequently calculated. During PASAT (+HS2), subjects simultaneously immersed their right foot in neutral water (33°C) to the level just above the malleolus to control for any change in body position or mechanical stimulation affecting the outcome (Fig 1).

CPT. During CPT (+HS2), the subjects were requested to immerse their right foot in ice water (1.2°C ± 1.3°C) to the level just above the malleolus for 5 minutes. All subjects performed the task without withdrawal. During CPT (+HS2), subjects simultaneously repeated the digits presented during the PASAT without any calculation to control for speech-induced respiratory changes affecting the outcome (Fig 1).

Control. In all sessions during HS1 and during HS2 in the control session, the subjects both performed the repetition of the digits presented during the PASAT without any calculation to control for speech-induced respiratory changes affecting the outcome and immersed their foot in neutral water to control for any change in body position or mechanical stimulation affecting the outcome (Fig 1).

Pain Assessment

Subjects reported HS-evoked pain intensity on a 0 to 10 numeric rating scale (NRS) indicating peak and average perceived pain levels after each infusion. "0" represented no pain and "10" represented maximum imaginable pain. Peak and average unpleasantness from the HS-evoked pain was also registered on a 0 to 10 NRS, where "0" represented no unpleasantness and "10" represented maximum imaginable unpleasantness.[21,27]

After each infusion, the subjects marked the spatial extent of the HS-evoked pain from an extraoral and an intraoral aspect on an anatomical drawing (DRAW). Subsequently DRAWs were digitized (Sigma Scan Pro 4.01.003) to obtain a quantitative measure of the pain area (mm^2) that included the potential referred pain area.[38,39]

Pain on palpation (POP) was estimated by means of a manual palpometer on a 0 to 100 NRS in which "0" was no sensation, "50" was just barely painful (pain detection threshold), and "100" was maximum imaginable pain. This scale was chosen to cover both nonpainful and painful sensations. At the beginning of each session, the subjects received careful and detailed instructions on how to rate the intensity of the mechanical stimulus, and it was ensured that the subjects understood the scale and the instructions.[40,41] POP levels were obtained on both the experimental and control (contralateral muscle) sides after each infusion. The manual palpometer consisted of a spring-coil with a 1-cm^2 probe by which 1 kg of pressure was applied to the central segment of the masseter muscle.[42] The choice of 1 kg pressure was made based on recommendations from the RDC/TMD.[32] Each manual palpation took approximately 2 seconds, on each side. Subjects were asked to keep their jaw and muscles in a relaxed position during palpation.

Autonomic Parameters

Throughout the entire session, the Task Force Monitor (TFM) (CNSystems Medizintechnik AG) noninvasively and continuously recorded the ECG, beat-to-beat and oscillometric blood pressure, impedance cardiography, and respiration (RESP).[4,43] From these recordings, mean values of HRV in the time and frequency domain, systolic and diastolic blood pressure (mmHg), stroke volume (mL), cardiac output (L/min), total peripheral resistance (dyne*s/cm^5), RESP (turns per minute [tpm]), and baroreceptor sensitivity (ms/mmHg) were estimated. Subjects acclimatized in the supine position for at least 30 minutes before TFM recordings. Four electrodes were placed for ECG monitoring. Three band-electrodes were placed for cardiographic monitoring, one situated at the neck and one at each side of the thorax at the level of processus xiphoideus. Finally, one ground electrode was placed at the right-side hip bone. All electrodes were original TFM single-use electrodes. Skin areas were disinfected prior to electrode application. Estimates of stroke volume were obtained from the impedance measurements.[44] An estimation of cardiac output was derived from stroke volume multiplied by heart rate. From the TFM default setting of the central venous pressure at 3 mmHg and the mean arterial blood pressure, total peripheral resistance was estimated as (mean arterial blood pressure minus

central venous pressure divided by cardiac output)*80. The rate of RESP was obtained from the band-electrodes at the xiphoid level.

Beat-to-beat blood pressure was recorded by the use of a double inflatable finger cuff measuring randomly at the second or third finger. Beat-to-beat blood pressure was automatically adjusted to the oscillometric blood pressure measured every fifth minute and from these measurements, systolic and diastolic blood pressures were generated. A measure of baroreflex activity was achieved from the mean slopes of all regression lines between RR-interval changes and systolic blood pressure levels.[45] RR-intervals are defined as the distance in ms between consecutive normal R waves from the QRS complexes in the ECG recordings.[46]

For estimation of HRV in the time and frequency domain, raw data from the ECG lead II was used. The TFM software is not suitable for detection and verification of the correctness of the QRS complexes.[46,47] In order to remove false detections due to noise or arrhythmias (ie, missing beats or ectopic beats), custom-made software was employed (Aalborg University). A Pan-Tompkins–like algorithm was used for QRS detection.[48] Power Spectral Analysis can be used to estimate autonomic tone from HRV.[49] Parasympathetic activity can be separated from sympathetic activity since high-frequency power (HF power; 0.15 to 0.4 Hz) is regarded as an index of pure cardiac vagal activity, and low-frequency power (LF power; 0.04 to 0.15 Hz) as a baroreflex-mediated response influenced by both parasympathetic and sympathetic activity.[29,46] HRV time domain measures were composed of mean RR interval, mean of all normal RR intervals (ms), standard deviation of all normal RR intervals (SDNN; ms), and the square root of the mean-squared differences of successive normal RR intervals (RMSSD; ms).[46] HRV frequency domain measures were composed of LF power (ms^2/Hz), coefficient of LF component variance (CCV-LF; %), HF power (ms^2/Hz), coefficient of HF component variance (CCV-HF, %), and total power (ms^2/Hz).[46] For power spectral analysis, the autoregressive method was used, with a model order of 20.[50] It was hypothesized that HRV, as a measure of autonomic function, during both stress tasks (CPT and PASAT) would be reduced but to a different extent and different from control.

Statistical Analyses

The number of subjects was based on a paired design sample size calculation. The objective was to be able to detect a 25% reduction in peak pain, and the intraindividual coefficient of variance of the peak pain measures was estimated to 20%, giving a minimum of 10 healthy subjects. Peak pain scores were the primary outcome parameter from the subject-based scores of pain. Average pain scores, peak and average unpleasantness scores, DRAW (intra- and extraoral), and POP scores were secondary effect parameters. Primary outcome parameters from the autonomic and cardiovascular measurements were mean RR, RMSSD, and SDNN. All other data were considered secondary outcome parameters. Absolute peak and average values of pain and unpleasantness scores were analyzed with the use of two-way analysis of variance (ANOVA) with session (CPT, PASAT, and control) and time (HS1 and HS2) as repeated measurement factors. Absolute values of DRAW, systolic and diastolic blood pressures, stroke volume, cardiac output, baroreceptor sensitivity, total peripheral resistance, and RESP were analyzed with the use of two-way ANOVA with session (CPT, PASAT, and control) and time (baseline 1, HS1, baseline 2, and HS2) as repeated measurement factors. POP scores were tested with the use of a three-way ANOVA with session, time, and side (experimental and control) as repeated measurement factors. To accommodate the assumptions of normal distributions, the HRV data in the frequency domain were log transformed before analysis. Mean RR intervals, SDNN, RMSSD, LF power, CCV-LF, HF power, CCV-HF, and total power were all tested with two-way ANOVA with session (CPT, PASAT, and control) and time (baseline 1, HS1, baseline 2, and HS2) as repeated-measurement factors. When appropriate, Tukey Honestly Significant Difference (Tukey HSD) test with corrections for multiple comparisons was used for post-hoc analyses. Possible correlations between pain reduction and percentages of correct answers at PASAT; pain reduction and blood pressure levels; pain reduction from PASAT and from CPT; and pain reduction and baroreceptor sensitivity were tested with Spearman rank correlation test. Data are presented as mean ± standard deviation (SD). Values of $P < .05$ were considered statistically significant.

Results

Pain Parameters

There was no main effect of session (CPT, PASAT, and control) in any of the primary or secondary pain parameters.

Primary Outcome Parameter. The mean HS-evoked peak pain score demonstrated a main effect of infusion (HS1 and HS2), significant session × infusion interaction, and significant within-session

Table 1 Results According to Pain and Unpleasantness (Peak and Average) Parameters (Summary of All Effects, ANOVA; Session × Time Interaction, Tukey HSD)

Parameters	Session	Infusion	Session × infusion interaction	Between-session differences	Within-session differences		
					CPT	PASAT	CTRL
Primary							
Peak pain	–	$P < .001$	$P < .001$	CPT < CTRL and PASAT < CTRL	HS1 > HS2	HS1 > HS2	–
Secondary							
Average pain	–	$P < .050$	$P < .050$	CPT < CTRL and PASAT < CTRL	HS1 > HS2	HS1 > HS2	–
Peak unpleasantness	–	$P < .050$	$P < .001$	CPT <CTRL and PASAT < CTRL	HS1 > HS2	HS1 > HS2	–
Average unpleasantness	–	$P < .050$	$P < .050$	–	HS1 > HS2	HS1 > HS2	–

Session × infusion interaction column is considered to reflect most important results. – indicates no significant difference; n = 14; values of $P < .05$ were considered statistically significant; CTRL = control.

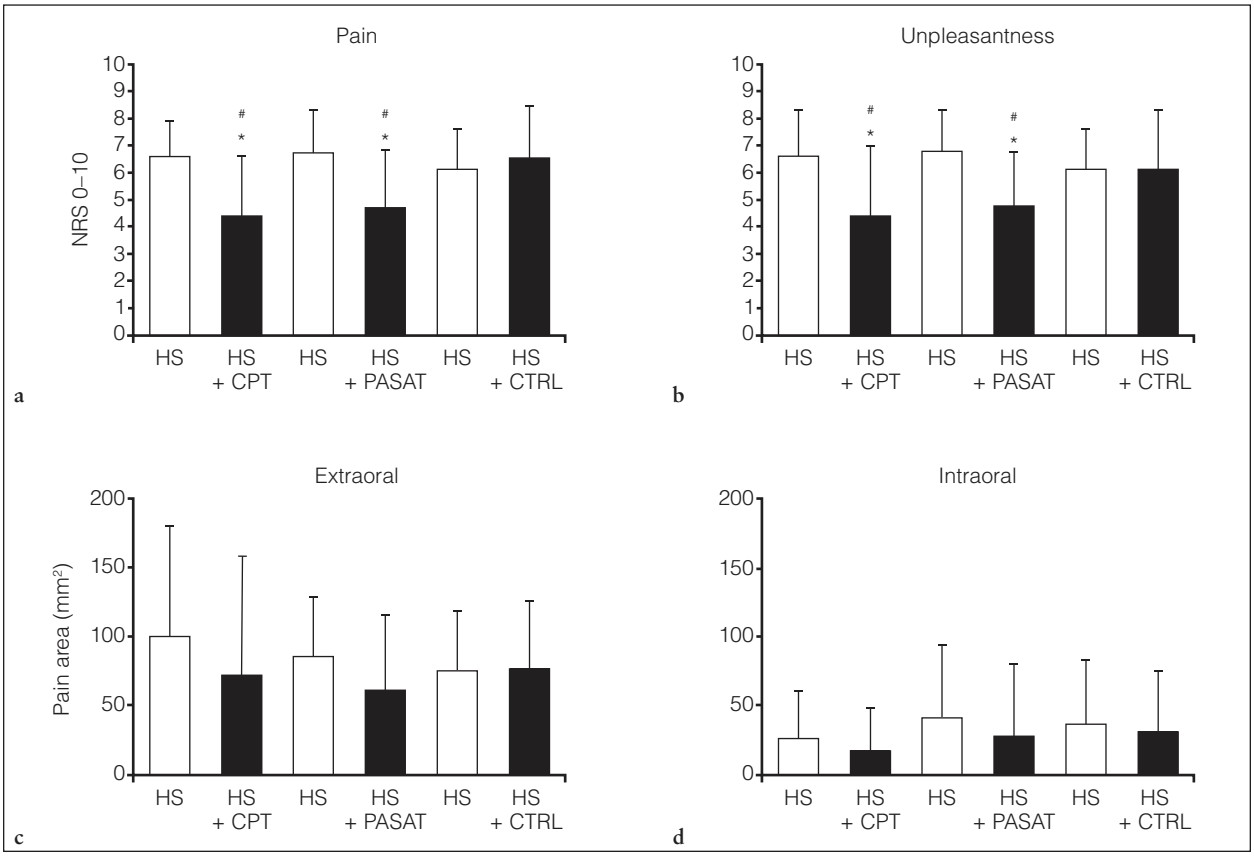

Fig 2 Pain, unpleasantness, and pain area drawing scores (means ± SD): *(a)* peak pain scores (0–10 NRS), *(b)* peak unpleasantness scores (0–10 NRS), *(c)* extraoral pain area drawing, *(d)* intraoral pain area drawing. $^{*}P < .05$ different from control; $^{\#}P < .05$ different from infusion 1; n = 14.

differences (Table 1, Fig 2a). The peak pain score was reduced by 35.9 ± 26.6% in the CPT session and by 30.8 ± 27.6% in the PASAT session compared with a relative increase in pain at the control session of 9.0 ± 30.5% (Table 1, Fig 2a).

Secondary Outcome Parameters. The mean HS-evoked average pain score demonstrated a main effect of infusion, significant session × infusion interaction, and significant within-session differences (Table 1). The mean HS-evoked peak and average

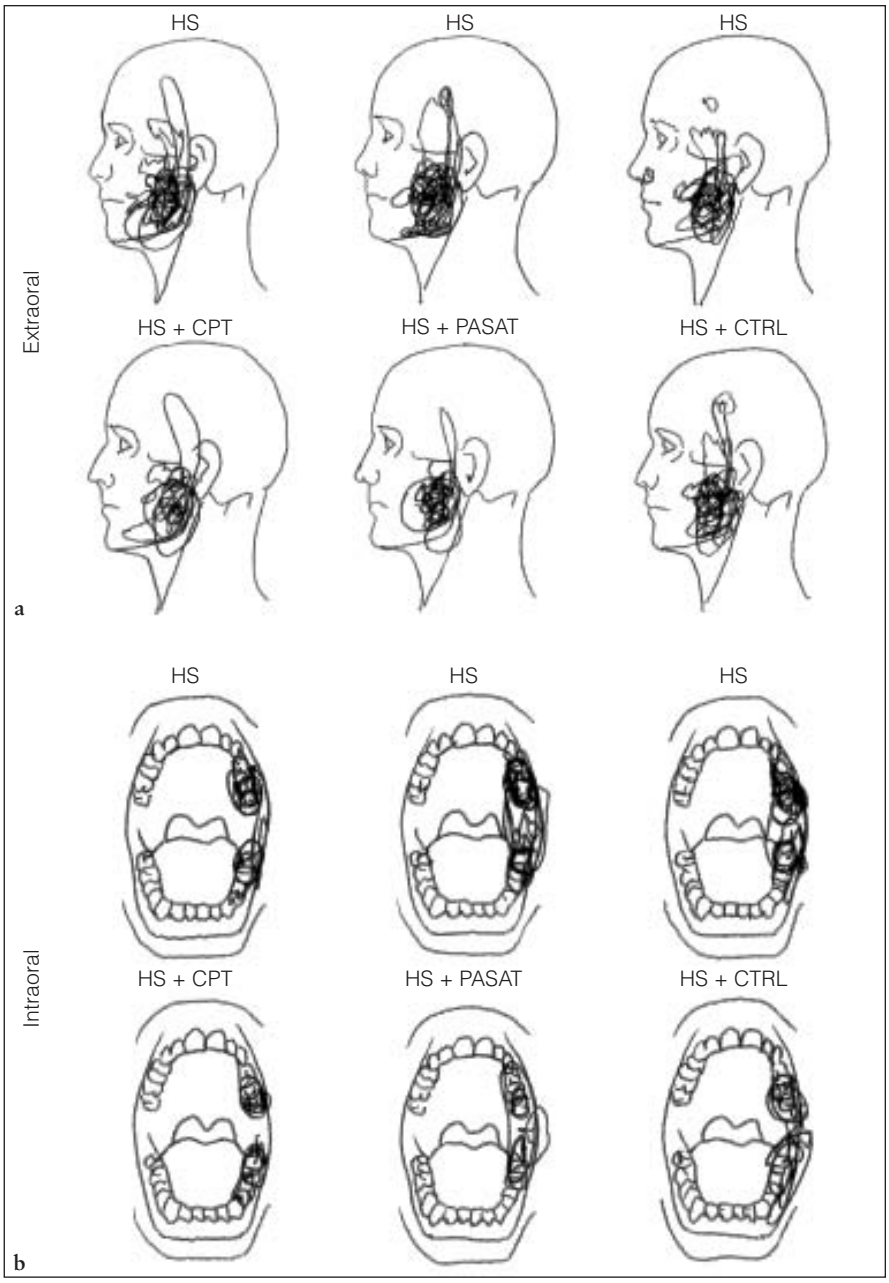

Fig 3 Illustrations of the subject-based drawings of the spatial extent of the HS-evoked masseter muscle peak pain from the two infusions in the three sessions. *(a)* extraoral perspective, *(b)* intraoral perspective; n = 14.

unpleasantness scores revealed main effects of infusion, significant session × infusion interactions, and significant within-session differences (Table 1 and Fig 2b).

The mean HS-evoked peak pain drawing area from both an extraoral and an intraoral perspective demonstrated a main effect of time (baseline 1, HS1, baseline 2, and HS2) (ANOVA: $P < .001$). A post-hoc test revealed that both the mean extra- and intraoral drawing areas from both infusions were significantly increased from both baseline areas (Tukey: $P < .021$). There were no differences between the mean baseline areas (Tukey: $P = .999$) and no differences between the mean HS1 and HS2 areas (Tukey: $P < .694$). No within-session differences were revealed between the HS1 and HS2 (Figs 2c, and 2d, Fig 3).

The mean POP score demonstrated no main effect of side (experimental side and control side) (ANOVA: $P = .283$). However, a main effect of time was revealed (ANOVA: $P < .001$). The post-hoc test demonstrated that the mean POP score at HS2 was significantly higher than the mean POP scores at both baselines (Tukey: $P < .034$). The mean POP score at HS1 was significantly higher than at baseline 1 (Tukey: $P = .008$), but it was not different from baseline 2 (Tukey: $P = .910$). There were no

Table 2 Results According to HRV and Hemodynamic Parameters (Summary of All Effects, ANOVA; Session × Time Interaction, Tukey HSD)

Parameters	Session	Time	Session × time interaction	Between-session differences	Within-session differences		
					CPT	PASAT	CTRL
Primary							
Mean RR (ms)	–	$P < .001$	$P < .001$	CPT < CTRL and PASAT < CTRL	HS1 > HS2	HS1 > HS2	–
RMSSD (ms)	–	$P < .050$	$P < .001$	CPT < CTRL and PASAT < CTRL	HS1 > HS2	–	–
SDNN (ms)	–	–	–	–	–	–	–
Secondary							
LF power (ms^2/Hz)	–	–	–	–	–	–	–
CCV-LF (%)	–	–	–	–	–	–	–
HF power (ms^2/Hz)	–	$P < .050$	$P < .050$	–	HS1 > HS2	–	–
CCV-HF (%)	–	–	$P < .050$	–	HS1 > HS2	–	–
Total power (ms^2/Hz)	–	–	$P < .050$	–	–	–	–
sBP (mmHg)	–	$P < .001$	–	–	–	–	–
dBP (mmHg)	–	$P < .001$	–	–	HS1 < HS2	–	–
SV (mL)	–	$P < .050$	$P < .001$	PASAT > CTRL	HS1 < HS2	–	–
CO (L/min)	–	$P < .001$	$P < .001$	CPT > CTRL and PASAT > CTRL	HS1 < HS2	HS1 < HS2	–
TPR (dyne*s/cm^5)	–	$P < .001$	$P < .050$	PASAT < CTRL	–	HS1 > HS2	–
RESP (tpm)	–	$P < .001$	$P < .050$	–	–	–	–
BRS (ms/mmHg)	–	$P < .050$	–	–	–	–	–

sBP = systolic blood pressure; dBP = diastolic blood pressure; SV = stroke volume; CO = cardiac output; TPR = total peripheral resistance; tpm = turns per minute; BRS = baroreceptor sensitivity. Session × Time Interaction column is considered to reflect most important results. – indicates no significant difference; values of $P < .05$ were considered statistically significant; n = 14.

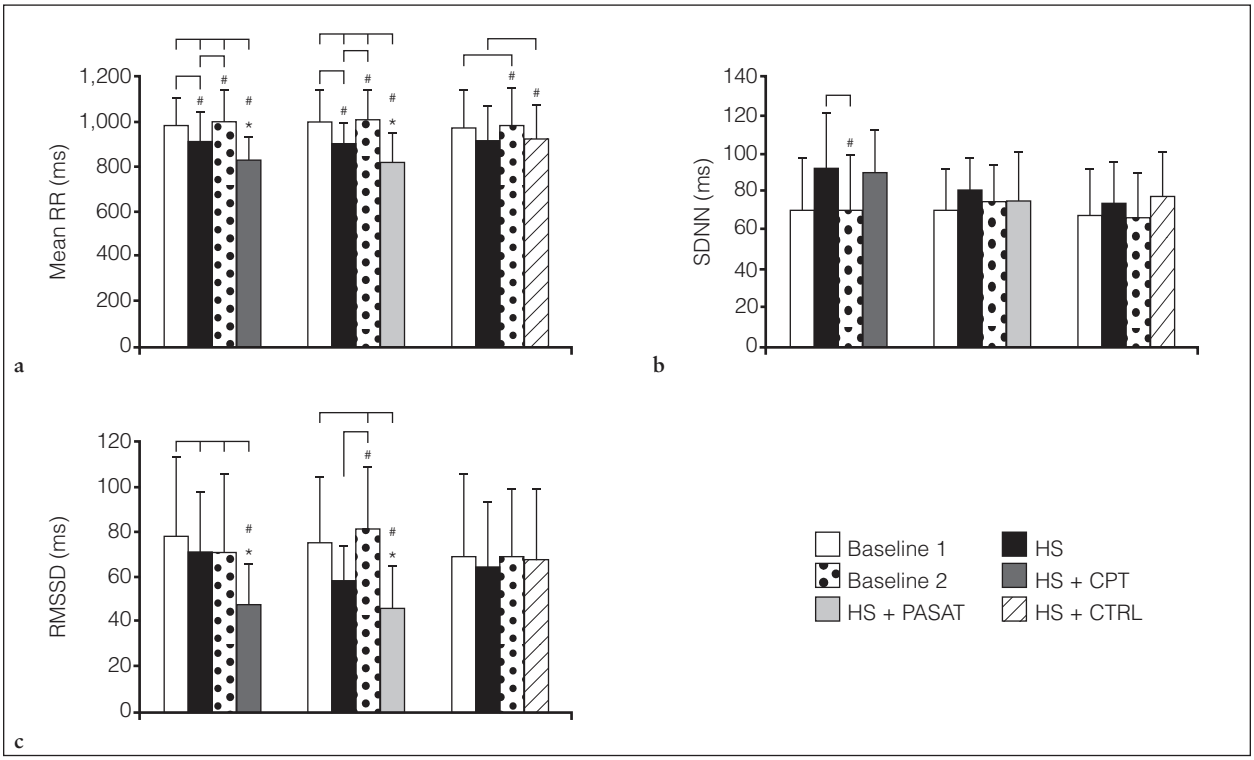

Fig 4 HRV measures in the time domain (means ± SD). Each group of four bars represent one session. *$P < .05$ different from control, #$P < .05$ within session; n = 14. Lines indicate significant differences.

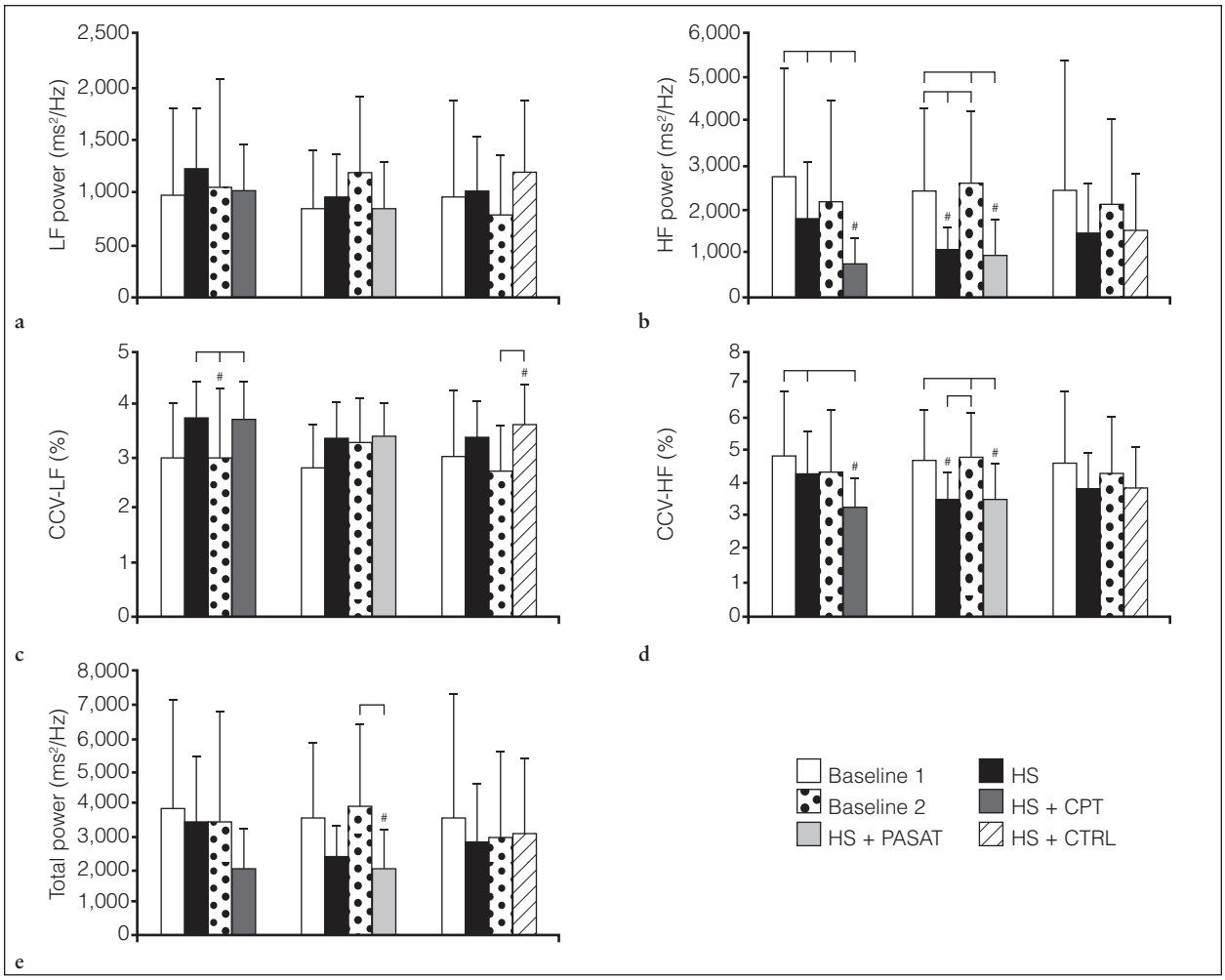

Fig 5 HRV measures in the frequency domain (means ± SD). HS: Each group of four bars represent one session. #$P < .05$ within session; n = 14. Lines indicate significant difference.

differences between the mean POP scores at baseline 1 and 2 (Tukey: $P = .052$) and between HS1 and HS2 (Tukey: $P = .156$). A significant side × time interaction was found (ANOVA: $P < .001$). The post-hoc test revealed no difference in POP scores at baseline 1 between sides (Tukey: $P = .999$). POP scores were significantly increased at the experimental side compared with the control side at HS1 (Tukey: $P < .001$), at baseline 2 (Tukey: $P = .037$), and at HS2 (Tukey: $P < .001$). The mean POP score did not at any time point reach the pain-detection threshold "50" on the 0 to 100 NRS.

HRV and Hemodynamic Parameters

A total of 19 incidents of artificial detections were deleted. In one of those incidents, the missing QRS complexes, after removal of an extrasystolic beat, were corrected by interpolation based on the previous three RR intervals.[46,51]

There was no main effect of session (CPT, PASAT, and control) in any of the primary or secondary HRV and hemodynamic outcome parameters.

Primary Outcome Parameters (HRV Measures in the Time Domain). In the mean RR interval, a main effect of time, a significant session × time interaction, and significant within-session differences were found (Table 2, Fig 4a). No main effect of time was detected in the mean SDNN, but significant within-session differences were found (Table 2, Fig 4b). For the RMSSD, a main effect of time, significant session × time interaction, and significant within-session differences were revealed (Table 2, Fig 4c).

Secondary Outcome Parameters. HRV Measures in the Frequency Domain. For the mean LF power and CCV-LF, no main effects of time were detected (Table 2, Fig 5a). In CCV-LF, significant within-session differences were found (Table 2, Fig 5c). For the mean HF power, a main effect of time, significant session × time interaction, and significant

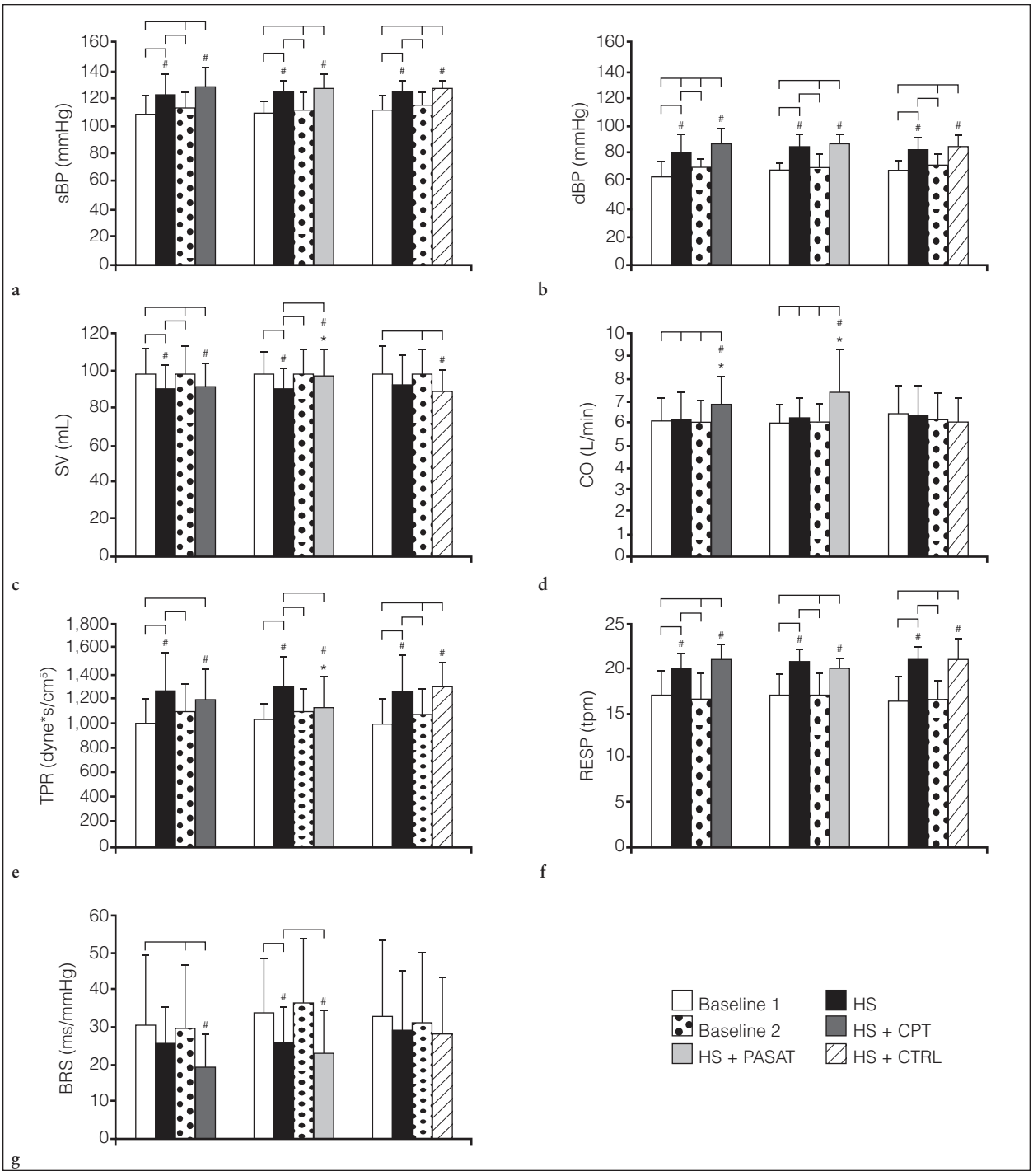

Fig 6 Hemodynamic parameters (means ± SD). Each group of four bars represent one session. *$P < .05$ different from control, #$P < .05$ within session; n = 14. Lines indicate significant differences.

within-session differences were found (Table 2, Fig 5b). No main effect of time was revealed in CCV-HF, but significant session × time interaction and significant within-session differences were found (Table 2, Fig 5d). In the mean total power, no main effect of time was detected, but both significant session × time interaction and significant within-session differences were revealed (Table 2, Fig 5e).

Hemodynamic Parameters. For both the mean continuous systolic and diastolic blood pressures,

main effects of time were revealed and significant within-session differences were found (Table 2, Figs 6a and 6b). In the mean stroke volume, mean cardiac output, mean total peripheral resistance, and RESP, main effects of time, significant session × time interactions, and significant within-session differences were found (Table 2, Figs 6c to 6f). For the mean baroreceptor sensitivity, a main effect of time and significant within-session differences were found (Table 2, Fig 6g).

Correlation Analyses

The mean percentages of correct answers at PASAT were 75.0 ± 14.8%. No correlation was found between PASAT scores and pain reduction ($\rho = 0.026$, $P = .929$).

Pain reduction from PASAT was inversely correlated to both systolic and diastolic ($\rho = -0.640$, $P = .014$; $\rho = -0.776$, $P = .001$) blood pressure levels. No correlation was found between pain reduction from CPT and these levels ($\rho = 0.145$, $P = .620$; $\rho = -0.065$, $P = .826$). The pain increase in the control session was significantly correlated to the rise in both levels ($\rho = 0.641$, $P = .013$; $\rho = 0.596$, $P = .024$).

No correlation was found between pain level changes from PASAT and CPT ($\rho = 0.277$, $P = .337$), or between pain level changes from PASAT and control ($\rho = 0.135$, $P = .646$), or between pain level changes from CPT and control ($\rho = 0.106$, $P = .719$).

Pain reduction from PASAT was correlated to baroreceptor sensitivity ($\rho = 0.600$, $P = .023$), whereas no correlation was found from baroreceptor sensitivity and pain reduction from CPT ($\rho = -0.232$, $P = .424$), or the pain increase in the control session ($\rho = 0.136$, $P = .644$).

Discussion

The primary findings of this randomized and controlled study were the significant and equivalent reduction of the experimental HS-evoked masseter muscle pain in healthy women from two different acute stressors, the PASAT and CPT. These findings were associated with significant and differential effects on autonomic system functions that possibly reflect involvement of different endogenous pain-modulatory systems.

Human Experimental Pain Model

The method of HS infusion into the masseter muscle of healthy subjects is a reliable and valid experimental pain model.[34,36,52] HS evokes nociceptor discharge and causes localized and referred muscle pain.[35,37] This experimental pain can to some extent mimic clinical muscle pain.[35,53] In the present study, the first infusion (HS1) in all three sessions was carried out as an internal control of the interindividual variation between sessions.[34] The HS-evoked pain was reliable, since there were no significant differences in VAS pain and unpleasantness scores between the internal control infusions (HS1 in the CPT, the PASAT, and the control sessions) and the control session (HS2+ CTRL). Therefore, the significant changes in HS-evoked pain during CPT and PASAT can be considered robust findings, strongly indicating activation of endogenous pain–modulatory systems.

One important observation from this study is the ability of the HS-evoked pain in the masseter muscle to induce a significant autonomic activation with increases in heart rate (primary outcome parameter), systolic and diastolic blood pressure, total peripheral resistance, and respiratory changes (secondary outcome parameters). This finding suggests that HS-evoked pain mimics real threat and that the normal physiological response to this stressor is a fight or flight reaction. Therefore, this experimental muscle pain model may indeed be valuable when studying the relationship between the autonomic nervous system and endogenous pain-modulatory pathways.

Pain Modulation

Pain reduction induced by CPT and PASAT were not correlated, suggesting involvement of different endogenous pain-modulatory systems. Indeed, HS-evoked jaw muscle pain applied together with a painful conditioning stimulus, ie, cold water, was expected to be decreased due to conditioned pain modulation, also known as diffuse noxious inhibitory control (DNIC). DNIC is a neurophysiological phenomenon examined in animal models in which nociceptive stimuli inhibit responses to another, but heterosegmental, nociceptive stimulus,[54–56] and conditioned pain modulation is the human counterpart where a painful conditioning stimulus inhibits pain evoked by painful stimuli applied to other body sites.[21,25,57,58] It was hypothesized that the decrease in HS-evoked jaw muscle pain during the mental stressor might be due to pain-modulatory mechanisms other than DNIC and conditioned pain modulation. Several studies have demonstrated that alterations in the psychological state can influence perceived pain in humans.[24,59] The arterial baroreceptors involved in the homeostatic control of blood pressure can be suppressed by psychological stress, and studies have demonstrated that reduced baroreflex sensitivity correlates with hypoalgesia.[60]

It should be noted that when the women illustrated on anatomical drawings the extent of their HS-evoked jaw muscle pain, neither CPT nor PASAT altered the extent of the areas compared with the control session. This measure of HS-evoked jaw muscle pain seems to be less sensitive to experimental manipulations than perceived intensity or unpleasantness. A slight mechanical sensitization was seen in the experimental masseter muscle after HS infusions compared with the control side. Despite this minor increased sensitivity, the mean POP values (secondary outcome parameter) never reached the pain-detection threshold, and no differences were detected from CPT and PASAT modulation. The lack of significant effects of CPT and PASAT on mechanical sensitization is probably due to a floor effect, ie, the magnitude of sensitization was too low to be influenced by the experimental conditions.

HRV

HRV was included as the primary outcome parameter for autonomic system function and is, indeed, regarded as a biomarker of how effectively an organism responds to stress as it reflects autonomic modulation.[29,46,61] A reduced HRV is considered a serious health risk and to have prognostic value in health and disease.[46,62] The literature describes findings of reduced HRV in patients suffering from chronic pain in fibromyalgia,[61,63] complex regional pain syndrome,[4] and TMD.[30]

In the present study, there was an increase in heart rate and a decrease in RMSSD (a vagal measure) during CPT compared with the internal control (first infusion), but also compared with the control session. All vagal measures (RMSSD, HF power, CCV-HF) were reduced, while the combined sympathetic and parasympathetic measures (LF power and CCV-LF) were unchanged. Therefore, the increased heart rate and reduced HRV during CPT was probably due to reduced parasympathetic activity. However, since this conclusion is partly based on secondary outcome parameters, this has to be interpreted with caution. The simultaneous application of two different painful stimuli (HS-evoked jaw muscle pain and CPT) may have resulted in an increased stress-state compared with only one pain stressor and thereby caused a potentially undesirable autonomic profile, ie, impaired HRV and vagal withdrawal. However, when it comes to the mental stressor, PASAT during HS-evoked jaw muscle pain, the increase in heart rate was not accompanied by a similar change in RMSSD when compared with the internal control (first infusion). In this case, the heart-rate increase probably cannot be explained by changes in sympathetic or parasympathetic activity to the heart, but instead it may be explained by sympathoadrenal release of catecholamines from adrenal medulla.[27] One finding that implies that the results should be interpreted with caution is the significant difference seen in RMSSD during PASAT compared with the control session; however, since there were no differences between the internal controls (HS1) and the control session (HS2-CTRL) in terms of RMSSD levels, the assumption of different modes of action from the two different acute stressors (CPT and PASAT) is supported.

Cardiovascular Response

One additional finding from this study that supports the hypothesis of activation of different endogenous pain-modulatory mechanisms due to CPT and PASAT was the inverse correlation between pain reduction from PASAT and the rise in blood pressure, whereas no correlations were present between pain reduction from CPT and blood pressure. The link between the rise in blood pressure and pain reduction from mental stress (PASAT), ie, a hypertension-related hypoalgesia, could be due to baroreceptor activation. Several studies suggest that the baroreceptors may mediate hypertension-related hypoalgesia.[15,60,64] The mechanism is not entirely known, but central neural regions involved in pain perception overlap significantly with cardiovascular regulatory areas.[18,60] The present data support the theory of baroreceptor involvement, since there was a positive correlation between baroreceptor sensitivity and pain reduction of HS-evoked jaw muscle pain during PASAT. The decrease in total peripheral resistance and the increase in stroke volume during PASAT would result in an increased blood flow to striated muscle tissue. This would change the arterial circulation and may thereby have affected the baroreceptors. Again these viewpoints are based on secondary outcome parameters and should be interpreted with caution, but the findings warrant further investigations.

There was no correlation in baroreceptor sensitivity and pain reduction of HS-evoked jaw muscle pain during CPT. This finding also supports the hypothesis that the endogenous pain-modulatory mechanisms of CPT and PASAT are different. During CPT and HS-evoked jaw muscle pain, an autonomic response similar to what has been seen in patients suffering from chronic pain was demonstrated. Several studies suggest that no baroreflex-mediated hypoalgesia exists in patients with chronic pain.[15,16,65,66]

Study Limitations

Obviously, caution should be taken when interpreting the results of the present study. Weaknesses include the relatively small sample size and the high number of statistical tests. However, the study had the advantage of using within-subject comparisons due to the paired study design,

Myofascial TMD are more prevalent in women than in men.[67] To eliminate the risk of potential sex differences in pain sensitivity[68] which may be caused by differences in the endogenous pain-inhibitory mechanisms,[57,58,69,70] and the possible sex differences in the autonomic response to stress-inducing factors,[71,72] only women were included in this study. Further studies will be needed to test for sex-related differences in PASAT- or CPT-induced effects in the present model.

The levels of circulating stress hormones were not assessed in the present study. These levels would have been valuable additional information in the differentiation of the pain-relieving mechanisms of CPT and PASAT. Another consideration is the difference between acute experimental pain in healthy subjects and chronic pain in patients[35,36,53] in terms of duration of pain, the psychological impact, disability, etc. It must also be considered to what extent the pain evoked by HS infusion into the masseter muscle mimics myofascial TMD, although some studies have suggested that the intensity, quality, and localization are sufficiently similar.[36,73] The outcome in this study is based on HS-evoked jaw muscle pain in healthy women, but women suffering from chronic myofascial TMD may respond differently to the application of stressors such as PASAT since the endogenous pain modulation appears to be altered in chronic pain patients.[13,74]

When interpreting the results of this study, the effect of cognitive distraction from both CPT and PASAT cannot be ignored, but it is not possible to separate the contribution of cognitive distraction from the results obtained. However, in the experimental set-up, efforts were made to make the two experimental conditions comparable; ie, during PASAT, subjects also placed their foot into neutral water, and during CPT, subjects repeated the same numbers as in the PASAT. Cognitive distraction is an effective method to reduce pain and in this study it is likely that the extent of distraction from both CPT and PASAT affected the results to a similar extent.

Conclusions

Application of CPT or PASAT reduced HS-evoked jaw muscle pain in this group of healthy women and altered the associated autonomic responses. However, the increase in heart rate following CPT and PASAT may have been caused by different mechanisms. It is suggested that CPT reduced the efferent cardiac vagal (parasympathetic) activity, whereas PASAT may have involved neurohumoral activation. Further studies are needed to examine similar endogenous pain-modulatory mechanisms in myofascial TMD pain patients.

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