Effect of Propranolol on Hypertonic Saline-Evoked Masseter Muscle Pain and Autonomic Response in Healthy Women During Rest and Mental Arithmetic Task

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Aims: *To investigate in a randomized, double-blinded, placebo-controlled, crossover study the effect of a single dose of the non-selective β-adrenergic receptor antagonist propranolol (40 mg) on hypertonic saline (HS)-evoked masseter muscle pain and autonomic activity during rest and during a mental arithmetic task (Paced Auditory Serial Addition Task, PASAT).* ***Methods:*** *Sixteen healthy women participated in two sessions in which propranolol or placebo was administered orally prior to two 5-minute infusions (30 minutes apart) of HS in the masseter muscle. The second HS infusion was combined with PASAT. HS-evoked pain intensity was scored on a numeric rating scale (NRS, 0 to 10). Heart rate variability and hemodynamic measures were recorded noninvasively (Task Force Monitor). Data were analyzed with repeated measurements analysis of variance (ANOVA).* ***Results:*** *Propranolol did not reduce NRS pain scores compared with placebo but did induce significant autonomic changes with reduced heart rate and increased heart rate variability (standard deviations of all normal RR intervals; root mean square successive differences; low-frequency power; high-frequency power; and total power) independent of the mental task.* ***Conclusion:*** *A single dose of propranolol had no effect on acute HS-evoked pain levels during rest or during mental arousal. However, it influenced the tone of the autonomic nervous system, possibly reflecting an anxiolytic effect.* J OROFAC PAIN 2013;27:243–255. doi: 10.11607/jop.1013

Key words: autonomic nervous system, experimental muscle pain, mental arithmetic task, propranolol, trigeminal nociceptio

Acute pain activates the autonomic nervous system with a series of physiological events including the release of the catecholamine stress hormones norepinephrine and epinephrine.[1] Elevated catecholamine levels have been reported in common chronic musculoskeletal pain conditions such as fibromyalgia[2] and myofascial temporomandibular disorders (TMD),[3] a highly prevalent subgroup of orofacial pain conditions that is overrepresented in women and characterized by pain and loss of function in the masticatory muscles.[4] The level of these two catecholamines depends both on the amount released and the rate of enzymatic degradation. Of the two enzymes responsible for catecholamine degradation, monamine oxidase (MAO) and catechol-O-methyltransferase (COMT), COMT activity appears to be most strongly related to persistent pain conditions.[5,6]

COMT activity in patients suffering from TMD appears to be reduced compared with pain-free controls.[7] Recently, several studies have also indicated an altered autonomic response in chronic myofascial TMD patients.[8–10] Nackley et al[6] demonstrated in rats increased nociceptive sensitivity due to inhibition of COMT and

thereby elevated catecholamine levels could be blocked by the nonselective β-adrenergic receptor antagonist propranolol and also by the combined administration of selective β_2- and β_3-adrenergic antagonists. This has led to the proposal that increased activation of β-adrenergic receptors might underlie muscle pain symptoms in common chronic musculoskeletal pain conditions. In patients with chronic TMD and low COMT activity, administration of propranolol was reported to reduce clinical pain levels.[11] Intravenous administration of propranolol also reduced clinical pain scores in a small number of TMD and fibromyalgia patients.[8] The mechanisms for the analgesic effect of propranolol in these common chronic musculoskeletal pain conditions is not known, but both central and peripheral mechanisms have been suggested.[12–15] Furthermore, it is unclear if the analgesic effect of a β-adrenergic antagonist is restricted to chronic muscle pain. For example, experimental pain evoked by serotonin injections into the human masseter muscle was reduced by local intramuscular administration of propranolol,[16] but whether oral administration of propranolol might also be effective in acute, nociceptive masticatory muscle pain from hypertonic saline (HS) infusions is not known. Oral drug administration is preferable from a patient comfort point of view compared with other routes of administration such as intravenous or intramuscular injection. Therefore, it would be of clinical benefit if propranolol, when used in the treatment of pain, could be orally administered.

Experimental masseter muscle pain evoked by 5% HS infusions is a reliable and valid experimental pain model.[17–20] HS evokes masseter muscle nociceptor discharge to cause localized and referred muscle pain,[21,22] mimicking many of the clinical manifestations of myofascial TMD pain[17,21] in addition to significant autonomic activation with increases in heart rate, systolic and diastolic blood pressure, and total peripheral resistance.[20] Due to the relatively constant and prolonged pain experience produced in healthy subjects with this model, it is optimal to evaluate changes in pain combined with changes in autonomic activity.

Chronic pain patients have an autonomic imbalance with reduced heart rate variability.[23] In healthy humans, arousal of the autonomic nervous system can be induced by a mental arithmetic task, the Paced Auditory Serial Addition Task (PASAT). The PASAT alters the autonomic activity in healthy human subjects by increasing heart rate and reducing heart rate variability.[20] This suggests that subjects simultaneously exposed to experimental pain and PASAT might manifest a reduced heart rate variability similar to that observed in chronic pain patients. Therefore, the aim of the present randomized, double-blinded, placebo-controlled crossover study was to investigate in a group of healthy women the effect of a single dose of the nonselective β-adrenergic receptor antagonist, propranolol (40 mg), on HS-evoked masseter muscle pain and autonomic function. The effect of propranolol was tested during conditions of rest and during PASAT. The following hypotheses were specifically tested: *(1)* propranolol reduces the intensity of HS-induced masseter muscle pain compared with placebo and *(2)* propranolol compared to placebo affects cardiovascular and other autonomic responses measured during HS-induced masseter muscle pain and during PASAT.

Materials and Methods

The project was registered within The Danish Data Protection Agency, Copenhagen, Denmark (No. 2008412790) and followed the guidelines of the World Medical Association Declaration of Helsinki, with thorough written and oral information about the experiment provided before subjects signed the informed consent document. The Central Denmark Region Committees on Biomedical Research Ethics (No. 20080183) approved the study.

The experiment was carried out on 18 healthy female volunteers (mean age 23.6, range 20 to 29 years), recruited from advertising at Aarhus University campus and at the webpage www.forsøgsperson.dk (comparable to www.sciencevolunteer.com). Volunteers reported no medication intake (except oral contraceptives); were not pregnant (subject-based report); reported no previous adverse reaction to β-adrenergic receptor antagonists, including hypersensitivity to propranolol or to any of its constituents; and had normal cardiovascular features as revealed on a 12-lead electrocardiogram (ECG). All participants completed the task but, due to technical failures, data from two women were subsequently excluded. Prior to inclusion, all participants were examined clinically by the same female investigator (KHB) to exclude TMD according to the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD).[24] All experimental sessions were performed by the same female investigator (KHB) at the Danish Pain Research Center, Aarhus University Hospital, Aarhus, Denmark.

The experiment was conducted as a double-blinded, placebo-controlled, randomized crossover study in which all subjects participated in two experimental sessions with a minimum interval of 7 days between the two sessions (Fig 1). Prior to each experimental session, participants were asked to abstain from

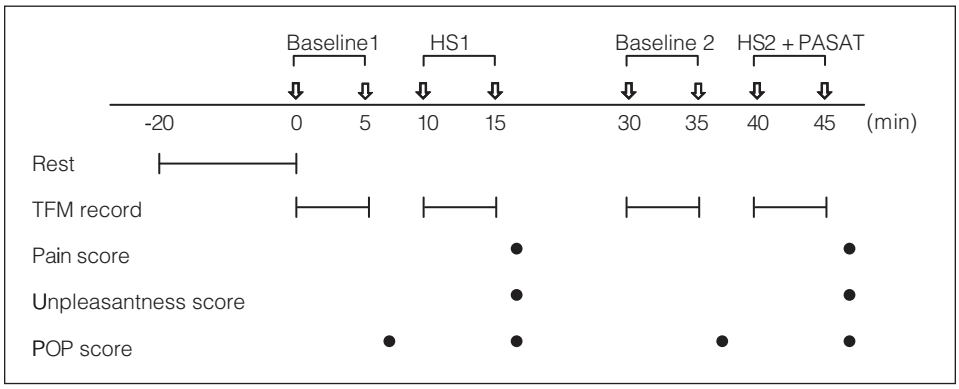

Fig 1 Illustration of the experimental protocol for both sessions. Propranolol 40 mg or placebo was taken 1.5 hours prior to Baseline 1. Baseline 1 = rest while task force monitor (TFM) recording, HS1 = first hypertonic saline (HS) infusion, Baseline 2 = rest while TFM recording, HS2+PASAT = second HS infusion with simultaneous performance of a mental arithmetic test (Paced Auditory Serial Addition Task, PASAT), Pain score = peak and average pain levels on a 0–10 numeric rating scale (NRS), Unpleasantness score = peak and average unpleasantness levels on a 0–10 NRS, POP score = pain on palpation levels on a 0–100 NRS. During HS1, the subjects were asked to repeat the digits from PASAT without calculation.

caffeinated beverages/foods and alcohol for at least 12 hours, to fast for a minimum of 2 hours, and to refrain from excessive physical activity and smoking for 12 hours (none of the participants reported to be regular smokers). One tablet of propranolol 40 mg (Propranolol "DAK", Nycomed Danmark Aps) or placebo (for blinding purposes, a tablet identical to propranolol in size, shape, and color) was taken orally 1.5 hours prior to baseline 1. This dose was based on the Tchivileva et al study, which reported that 20 mg of propranolol twice a day (40 mg/day) for 1 week reduced clinical pain levels.[11] Subjects were randomly assigned to a treatment order, either propranolol in the first session followed by placebo in the second session or placebo in the first session followed by propranolol in the second session, by the use of a computer. Investigator and subjects were unaware of the treatment order. All experimental sessions were performed in a standardized manner, with a duration of approximately 1.5 hours, and took place in a quiet room with a temperature of about 23°C. Participants were positioned supine and not allowed to speak during experimental recordings, unless an emergency situation occurred (Fig 1) or when performing the PASAT (see below).

HS-Evoked Pain

Sterile HS was infused into the deep central portion of the left masseter muscle (experimental side) to evoke muscle pain.[17–19] Each infusion was administered through a 27-G hypodermic needle and disposable syringe, and by the use of a B. Braun Perfusor Space syringe pump as described in detail in Bendixen et al.[20] Within each experimental session, two infusions of 5 minutes duration were given, 30 minutes apart. During each infusion, a bolus of 0.14 mL HS (infusion rate 51.42 mL/hour) was initially administered followed by a maintenance infusion rate of 6 mL/hour for 5 minutes. In total, approximately 0.60 mL HS was administered per infusion. The first infusion (HS1) served as an internal control for variations between sessions[19,20,25] and as a control infusion within session. During the second infusion, PASAT was simultaneously performed. The second infusion is referred to as HS2+PASAT.

Paced Auditory Serial Addition Task

The PASAT, performed during the second infusion in both sessions, was a random sequence of digits from one to nine with a constant interval of 2.4 seconds between each digit[26] presented to the subjects through headphones. The task was to add the last two presented digits continuously and immediately report the sum out loud for a period of 5 minutes duration.[20,26,27] The subjects were requested to concentrate, to score as many correct answers as possible, and to resume calculations immediately if an incorrect answer was given or an interruption occurred. Subsequently (and unknown to the authors), the percentage of correct answers in total for the 5-minute task was calculated.[20,27]

To control for speech-induced respiratory changes affecting the outcome, during HS1, the digits from PASAT were presented in the same manner for the

entire 5-minute duration, but instead the subjects were asked to repeat the digits out loud without calculation (Fig 1).

Pain Measurement

Subjects reported the intensity of HS-evoked pain and unpleasantness on separate 0–10 NRSs indicating peak and average perceived pain and unpleasantness levels following each infusion. "0" represented no pain/unpleasantness and "10" maximum imaginable pain/unpleasantness.[20,27,28]

Pain on palpation (POP) was estimated by means of a manual palpometer consisting 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.[20,29] The choice of 1 kg pressure was made based on recommendations from the RDC/TMD.[24] Pain was rated on a 0–100 NRS in which "0" was no sensation, "50" just barely painful (pain detection threshold), and "100" maximum imaginable pain.[30,31] This scale was chosen to cover both non-painful 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.[29,31] POP levels were obtained on both the experimental (left side masseter muscle) and control (right side masseter muscle) sides after each infusion. Each manual palpation on each side took approximately 2 seconds. Subjects were asked to keep their jaw and muscles in a relaxed position during palpation.

Autonomic Parameters

Throughout both entire sessions, the Task Force Monitor (TFM) (CNSystems Medizintechnik AG) noninvasively and continuously recorded the ECG, beat-to-beat blood pressure, impedance cardiography, and respiration (RESP).[20,23,32] From these recordings, mean values of heart rate variability in the time and frequency domain, systolic and diastolic blood pressure (sBP/dBP; mmHg), stroke volume (SV; mL), cardiac output (CO; L/min), total peripheral resistance (TPR; dyne*s/cm^5), RESP (breath/min), and baroreceptor sensitivity (BRS; ms/mmHg) were estimated. See Terkelsen et al[23] for further details. Subjects were acclimatized to the setup in supine position for 30 minutes before TFM recordings.

For estimation of heart rate variability in the time and frequency domain, raw data from ECG lead II was used. All ECG recordings were manually inspected. To remove false detections due to noise or arrhythmias (ie, missing beats or ectopic beats), custom-made software was employed (Aalborg University, Denmark). A Pan-Tompkins–like algorithm was used for QRS detection.[33] Each of the 16 subjects had two sessions of ECG recordings, and in two out of 32 sessions, two extrasystolic beats were corrected by interpolation based on the previous three RR intervals.

Heart rate variability expressed in the time domain was: mean of all normal RR intervals (mean RR interval; 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).[34] Heart rate variability expressed in the frequency domain was: low frequency power (LF-power; ms^2/Hz), coefficient of LF component variance (CCV-LF;%), LF power normalized to total power (LF-norm; nu), high-frequency power (HF-power; ms^2/Hz), coefficient of HF component variance (CCV-HF; %), HF power normalized to total power (HF-norm; nu), LF-power/HF-power ratio (LF/HF-ratio; %), and total power (ms^2/Hz). For power spectral analysis, an autoregressive method was used, with a model order of 20.[34]

Statistical Analyses

The number of subjects was based on a paired-design sample-size calculation that could detect a 25% reduction in peak pain. The intraindividual coefficient of variance of the peak pain measures was estimated to be 20%, which indicated that a minimum of 10 healthy subjects would be required. The primary outcome parameter from the subject-based scores was peak pain. Primary outcome parameters from the autonomic and cardiovascular assessments were mean RR intervals, SDNN, and RMSSD. All other data collected were regarded as 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 treatment (propranolol and placebo) and time (HS1 and HS2+PASAT) as repeated measurement factors. Absolute values of sBP, dBP, SV, CO, TPR, RESP, BRS, mean RR intervals, SDNN, RMSSD, LF-power, CCV-LF, LF-norm, HF-power, CCV-HF, HF-norm, LF/HF-ratio, and total power were analyzed with the use of two-way ANOVA with treatment (propranolol and placebo) and time (Baseline 1, HS1, Baseline 2, and HS2+PASAT) as repeated measurement factors. POP scores were tested with the use of a three-way ANOVA with treatment, time, and side (experimental and control) as

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

Parameter	Treatment	Infusion	Treatment × Infusion interaction	Between-treatment differences		Within-treatment differences	
				HS1	HS2+PASAT	Propranolol	Placebo
Primary							
Peak pain		$P < .001$				HS2+PASAT ↓	HS2+PASAT ↓
Secondary							
Average pain		$P < .001$				HS2+PASAT ↓	HS2+PASAT ↓
Peak unpleasantness		$P < .050$				HS2+PASAT ↓	HS2+PASAT ↓
Average unpleasantness		$P < .050$				HS2+PASAT ↓	HS2+PASAT ↓

HS1 = first hypertonic saline infusion within treatment and HS2 = second hypertonic saline infusion within treatment. Pain and unpleasantness parameters were scored on a 1–10 numeric rating scale. PASAT = Paced Auditory Serial Addition Task. ANOVA = analysis of variance (two-way). Tukey HSD = Tukey Honestly Significant Difference test. Highlighted column, Treatment × Infusion interaction, is considered the most important result. Spaces left empty indicate no significant differences. n = 16, values of $P < .05$ were considered statistically significant.

repeated measurement factors. To accommodate the assumptions of normal distributions, the heart rate variability data in the frequency domain were log transformed before analysis. When appropriate, the Tukey Honestly Significant Difference (Tukey HSD) test with corrections for multiple comparison was used for post-hoc analyses. A paired *t* test was used to analyze the difference in the percentage of correct answers in the PASAT score between treatments. All results are presented as means ± SD. Values of $P < .05$ were considered statistically significant.

Results

Pain Parameters

There was no main effect of treatment (propranolol or placebo) in any of the primary or secondary pain parameters (Table 1).

Primary Outcome Parameters. The mean HS-evoked peak pain score demonstrated a main effect of infusion (HS1 and HS2+PASAT) (ANOVA: $df = 1$; $F = 20.43$; $P < .001$). The post-hoc test revealed that the peak pain during the second infusion, when at the same time the subjects were performing PASAT, was significantly lower than during the first infusion without performing PASAT (Tukey: $P < .010$). Significant within-treatment differences were revealed (Fig 2 and Table 1). HS evoked peak pain levels were, in all subjects, moderate to strong (NRS 6.7 ± 1.2).

Secondary Outcome Parameters. The mean HS-evoked average pain score and the mean peak and average unpleasantness scores revealed a main effect of infusion (ANOVA: $df = 1$; $F > 14.27$; $P < .002$). Significant within-treatment differences were revealed (Fig 2 and Table 1).

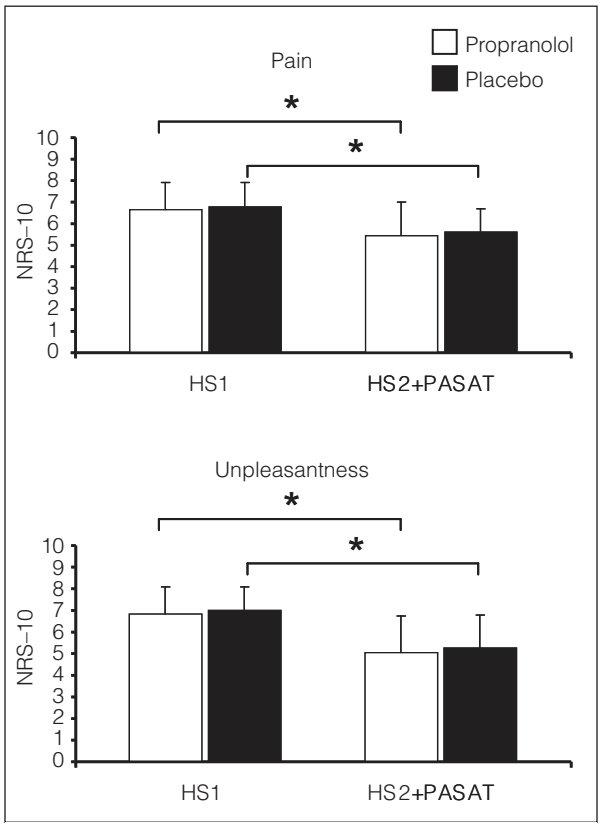

Fig 2 Pain and unpleasantness scores (means ± SD). HS1 = first hypertonic saline (HS) infusion, HS2+PASAT = second HS infusion with simultaneously performance of the Paced Auditory Serial Addition Task (PASAT). NRS = numeric rating scale. n = 16, $*P < .05$.

The mean POP scores revealed no main effect of side (experimental side and control side) (ANOVA: $df = 1$; $F = 1.90$; $P = .178$), but a main effect of time was found (ANOVA: $df = 3$; $F = 13.30$; $P < .001$). A post-hoc test revealed that the mean POP scores at HS1, Baseline 2, and HS2+PASAT were

Table 2 Results According to Heart Rate Variability and Hemodynamic Parameters (Summary of All Effects, ANOVA; Treatment × Time Interaction, Tukey HSD)

			Treatment × Time interaction	Between-treatment differences		Within-treatment differences	
	Treatment	Time		HS1	HS2 + PASAT	Propranolol	Placebo
Heart rate variability measures							
Primary parameters							
Mean RR (ms)	$P < .001$	$P < .001$	$P < .001$	PROP↑	PROP↑	HS2+PASAT↓	HS2+PASAT↓
SDNN (ms)	$P < .050$	$P < .050$	$P < .050$	PROP↑	PROP↑		
RMSSD (ms)	$P < .050$		$P < .001$	PROP↑	PROP↑		
Secondary parameters							
LF-power (ms^2/Hz)	$P < .050$			PROP↑	PROP↑		
CCV-LF (%)							
LF-norm (nu)		$P < .001$	$P < .050$				
HF-power (ms^2/Hz)	$P < .050$		$P < .001$	PROP↑	PROP↑		HS2+PASAT↓
CCV-HF (%)			$P < .050$	PROP↑	PROP↑		
HF-norm (nu)		$P < .001$	$P < .050$		PROP↑		
Total power (ms^2/Hz)	$P < .050$		$P < .050$	PROP↑	PROP↑		
LF/HF ratio (%)		$P < .001$	$P < .050$		PROP↓		
Hemodynamic parameters							
Secondary parameters							
BRS (ms/mmHg)	$P < .050$	$P < .050$					
Resp (breath/min)	$P < .050$	$P < .001$	$P < .050$	PROP↓			
sBP (mmHg)	$P < .050$	$P < .001$					
dBP (mmHg)		$P < .001$					
SV (mL)	$P < .050$	$P < .001$	$P < .001$	PROP↓	PROP↓		
TPR (dyne*s/cm^5)	$P < .001$	$P < .001$	$P < .001$	PROP↑	PROP↑		
CO (L/min)	$P < .001$	$P < .050$	$P < .001$	PROP↓	PROP↓		HS2+PASAT↑

Heart rate variability measures: Mean RR, mean of all normal RR intervals; SDNN, standard deviation of all normal RR intervals; RMSSD, the square root of the mean-squared differences of successive normal RR intervals; LF-power, low-frequency power; CCV-LF, coefficient of LF component variance; LF-norm, low-frequency power normalized to total power; HF-power, high-frequency power; CCV-HF, coefficient of HF component variance; HF-norm, high-frequency power normalized to total power; LF/HF ratio, normalized low-frequency power divided by normalized high-requency power. Hemodynamic parameters: BRS, baroreceptor sensitivity; Resp, breath/minute; sBP, systolic blood pressure; dBP, diastolic blood pressure; SV, stroke volume; TPR, total periferal resistance; CO, cardiac output. HS1 = first hypertonic saline infusion within treatment and HS2 = second hypertonic saline infusion within treatment. PASAT = Paced Auditory Serial Addition Task. PROP = propranolol 40 mg. PROP ↑/↓ = treatment with propranolol significantly higher/lower than placebo. HS2+PASAT ↑/↓ = second hypertonic saline infusion + PASAT significantly higher/lower than first hypertonic saline infusion (HS1). ANOVA = analysis of variance (two-way). Tukey HSD = Tukey Honestly Significant Difference test. Highlighted column, Treatment × Time interaction, is considered the most important result. Spaces left empty indicate no significant differences. n = 16. Values of $P < .05$ were considered statistically significant.

all significantly higher than at Baseline 1 (Tukey: $P < .014$), and that POP scores at HS2+PASAT were significantly higher than HS1 and Baseline 2 (Tukey: $P < .016$). There was no difference between HS1 and Baseline 2 (Tukey: $P = .998$). No side X treatment interaction was revealed (ANOVA: $df = 1$; $F = 3.14$; $P = .087$); however, a significant side X time interaction was found (ANOVA: $df = 3$; $F = 10.16$; $P < .001$). The post-hoc test revealed no difference in POP scores at Baselines 1 and 2 between sides (Tukey: $P > .114$); however, the POP scores were significantly increased on the experimental side compared with the control side at both HS1 and HS2+PASAT (Tukey: $P < .022$), yet the mean POP score did not at any time point reach the pain-detection threshold "50" on the 0–100 NRS.

Heart Rate Variability and Hemodynamic Parameters

Primary Outcome Parameters. Heart Rate Variability Measures in the Time Domain. The mean RR

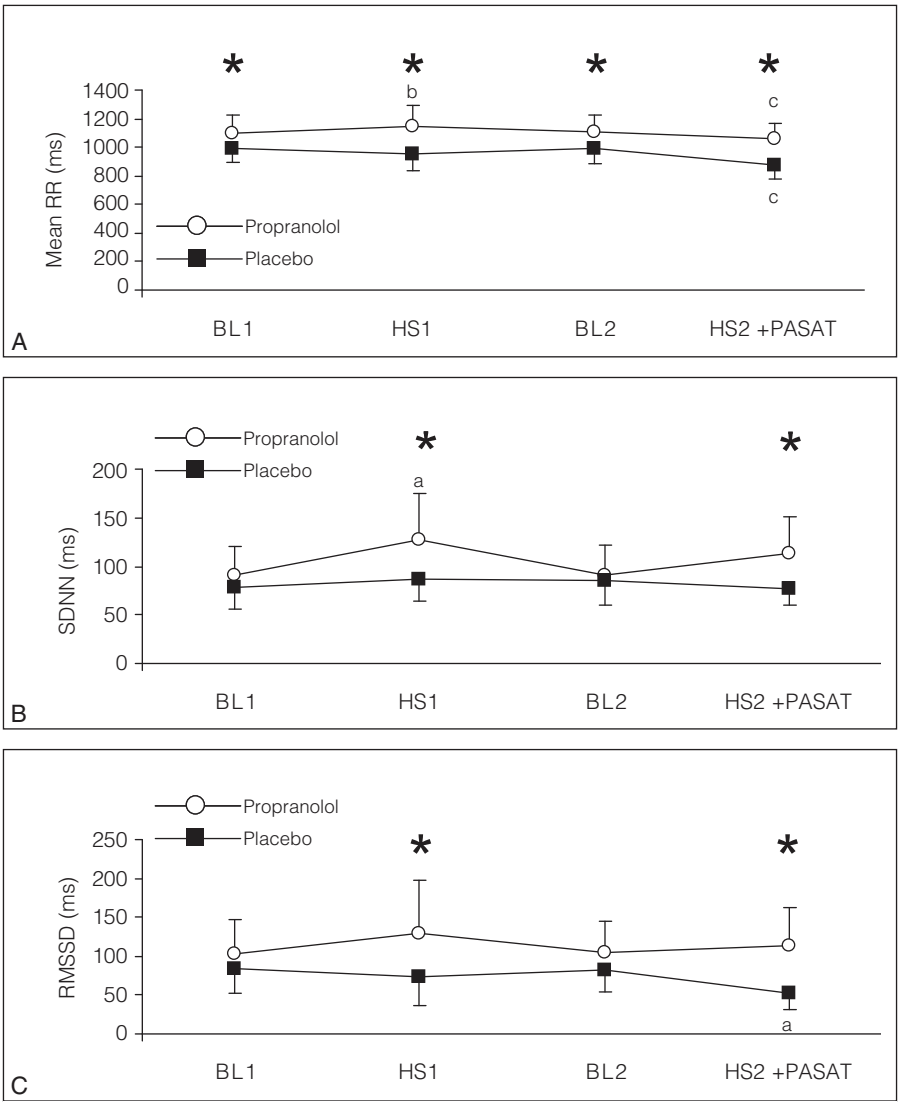

Fig 3 Heart rate variability measures in the time domain (means ± SD). (A) mean RR, mean of all normal RR intervals. (B) SDNN, standard deviation of all normal RR intervals. (C) RMSSD, the square root of the mean-squared differences of successive normal RR intervals. HS1 = first hypertonic saline (HS) infusion, HS2+PASAT = second HS infusion with simultaneous performance of the Paced Auditory Serial Addition Task (PASAT). BL1 and BL2 = Baseline 1 and 2. n = 16. *$P < .05$ propranolol different from placebo. Within-session differences: a = $P < .05$ different from both BL1 and BL2; b = $P < .05$ different from BL1; c = $P < .05$ different from BL1, HS1, and BL2.

interval data analysis demonstrated a main effect of treatment (ANOVA: $df = 1$; F = 48.99; $P < .001$). A Tukey post-hoc test revealed that propranolol treatment resulted in significantly longer mean RR intervals compared with placebo treatment (Tukey: $P < .001$). A main effect of time was also demonstrated (ANOVA: $df = 3$; F = 11.43; $P < .001$). The post-hoc test revealed that the mean RR interval during HS2+PASAT was significantly shorter than during Baseline 1, HS1, and Baseline 2 (Tukey: $P < .001$). A significant treatment × time interaction also was found (ANOVA: $df = 3$; F = 8.86; $P < .001$). Post-hoc test revealed significantly longer mean RR intervals at all time points (Baseline 1, HS1, Baseline 2, and HS2+PASAT) during treatment with propranolol compared with placebo, respectively (Tukey: $P < .001$) (Table 2 and Fig 3, A).

Analysis of the mean SDNN data revealed a main effect of treatment (ANOVA: $df = 1$; F = 7.44; $P < .016$). A Tukey post-hoc test revealed that propranolol treatment resulted in significantly larger SDNN compared with placebo treatment (Tukey:

$P < .016$). A main effect of time was also demonstrated (ANOVA: $df = 3$; $F = 3.24$; $P < .031$). The post-hoc test revealed that SDNN during HS1 was significantly larger than during Baseline 1 (Tukey: $P < .025$). Also a significant treatment × time interaction was found (ANOVA: $df = 3$; $F = 2.90$; $P < .001$). A post-hoc test revealed significantly larger SDNN at HS1 and HS2+PASAT during treatment with propranolol compared with placebo, respectively (Tukey: $P < .009$) (Table 2 and Fig 3, B).

The mean RMSSD data analysis demonstrated a main effect of treatment (ANOVA: $df = 1$; $F = 11.92$; $P < .004$). A Tukey post-hoc test revealed that propranolol treatment resulted in significantly larger RMSSD compared with placebo treatment (Tukey: $P < .004$). No main effect of time was revealed (ANOVA: $df = 3$; $F = 1.92$; $P < .140$). A significant treatment × time interaction was demonstrated (ANOVA: $df = 3$; $F = 7.93$; $P < .001$). A post-hoc test revealed significantly larger RMSSD at HS1 and HS2+PASAT during treatment with propranolol compared with placebo, respectively (Tukey: $P < .001$) (Table 2 and Fig 3, C).

Secondary Outcome Parameters. Heart Rate Variability Measures in the Frequency Domain. The mean LF-power data analysis demonstrated a main effect of treatment, but no main effect of time was found (Table 2 and Fig 4, A). CCV-LF data revealed no main effect of treatment or time (Table 2 and Fig 4, E). The mean LF-norm data demonstrated no main effect of treatment, but a main effect of time and treatment × time interaction was revealed (Table 2 and Fig 4, C).

Analysis of the mean HF-power data revealed a main effect of treatment, but no main effect of time was found. A significant treatment × time interaction was also demonstrated (Table 2 and Fig 4, B). The mean CCV-HF data demonstrated no main effect of treatment or time, but a significant treatment × time interaction was found (Table 2 and Fig 4, F). There was no main effect of treatment detected in the mean HF-norm. A main effect of time and significant treatment × time interaction was found (Table 2 and Fig 4, D). The mean LF/HF ratio data revealed no main effect of treatment, but both a main effect of time and a significant treatment × time interaction was found (Table 2 and Fig 4, G). The mean total power demonstrated a main effect of treatment but no main effect of time. A significant treatment × time interaction was also detected (Table 2 and Fig 4, H).

Hemodynamic Parameters. Analysis of the mean sBP data demonstrated main effects of treatment and time, whereas no main effect of treatment was revealed in the mean dBP data (ANOVA: $df = 1$; $F = 2.26$; $P = .154$) but a main effect of time (Table 2 and Fig 5, A and B).

Main effects of treatment, time, and significant treatment × time interactions were demonstrated in the mean SV, CO, TPR, and RESP rate (Table 2 and Fig 5, C and F). The mean BRS data revealed a main effect of treatment (ANOVA: $df = 1$; $F = 7.98$; $P = .013$) and time (ANOVA: $df = 3$; $F = 4.80$; $P = .006$) (Table 2 and Fig 5, G).

PASAT Score

The percentage correct answers when performing PASAT during propranolol treatment 78.0% ± 11.4% did not differ from placebo 75.8% ± 9.8% (paired *t* test: $P = .555$).

Discussion

In the present randomized and placebo-controlled study in a group of healthy women, propranolol induced significant autonomic changes with reduced heart rate and increased heart rate variability. However, propranolol did not have any analgesic effect, either during rest or during mental arousal.

Experimental Pain Modulation

The present human experimental pain model revealed that infusion of HS into the masseter muscle of healthy subjects is a reliable and valid experimental pain model, which to some extent shares features with and thereby resembles clinical muscle pain.[17,18] In the present study, the chosen model was confirmed to be appropriate for studying pain and autonomic nervous system interactions, because HS consistently, in all subjects, evoked moderate to strong levels of pain (mean NRS peak pain 6.7 ± 1.2) in addition to robust autonomic responses such as increases in heart rate, sBP/dBP, SV, and RESP.

Propranolol (propranolol hydrochloride) is a nonselective β-adrenergic receptor antagonist without partial agonist effects and is primarily used in the treatment of cardiovascular diseases, tremor, and migraine prophylaxis, but also in certain anxiety disorders such as those characterized by somatic symptoms and by performance anxiety.[35] The significant increase in mean RR interval and significant increase in TPR during propranolol confirms that β-adrenoceptor blockade was achieved both at the cardiac and vascular smooth muscle level. However, HS-evoked pain level from the first infusion during treatment with propranolol 40 mg did not differ from placebo.

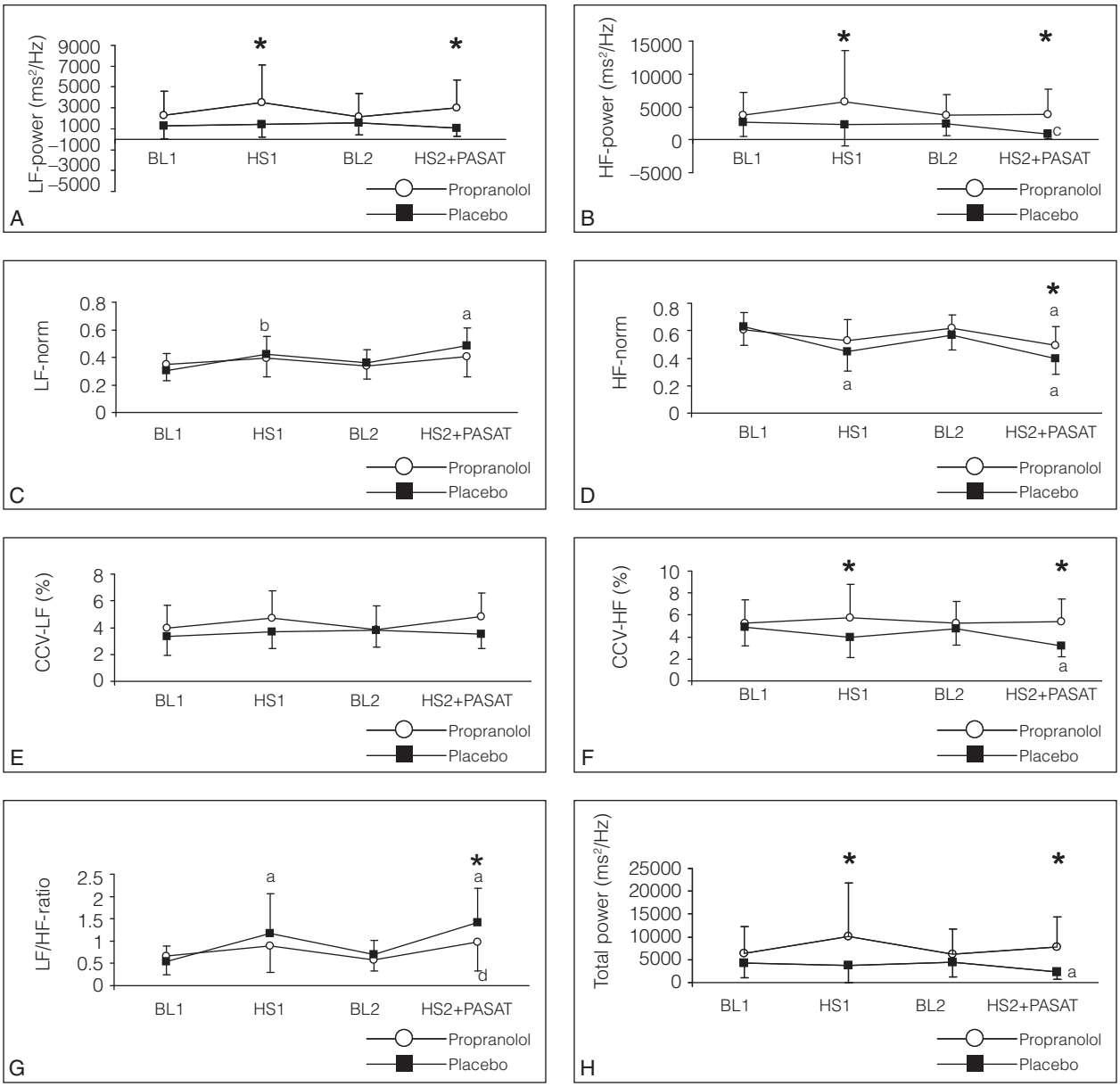

Fig 4 Heart rate variability measures in the frequency domain (means ± SD). (A) LF-power, low-frequency power. (B) HF-power, high-frequency power. (C) LF-norm, low-frequency power normalized to total power. (D) HF-norm, high-frequency power normalized to total power. (E) CCV-LF, coefficient of LF component variance. (F) CCV-HF, coefficient of HF component variance. (G) LF/HF ratio, normalized low-frequency power divided by normalized high-frequency power. (H) Total power. HS1 = first hypertonic saline (HS) infusion, HS2+PASAT = second HS infusion with simultaneous performance of the Paced Auditory Serial Addition Task (PASAT). BL1 and BL2 = Baseline 1 and 2. n = 16. *$P < .05$ propranolol different from placebo. Within-session differences: a = $P < .05$ different from both BL1 and BL2; b = $P < .05$ different from BL1; c = $P < .05$ different from BL1, HS1, and BL2; d = $P < .05$ different from BL2.

The mechanisms by which propranolol induces analgesia, when COMT activity is low and catecholamine levels are high,[11] are not clear, and both peripheral and central neural mechanisms have been suggested.[12,15,36] It has been demonstrated that propranolol has membrane-stabilizing effects and, like conventional local anesthetics such as lidocaine, blocks sodium channels.[15] However, it is not likely that the dose applied in this study could have resulted in sodium channel block, since it requires a relatively high drug concentration to observe such effects. The anxiolytic properties of propranolol are well described in the literature and reflect one of the central mechanisms suggested to explain why

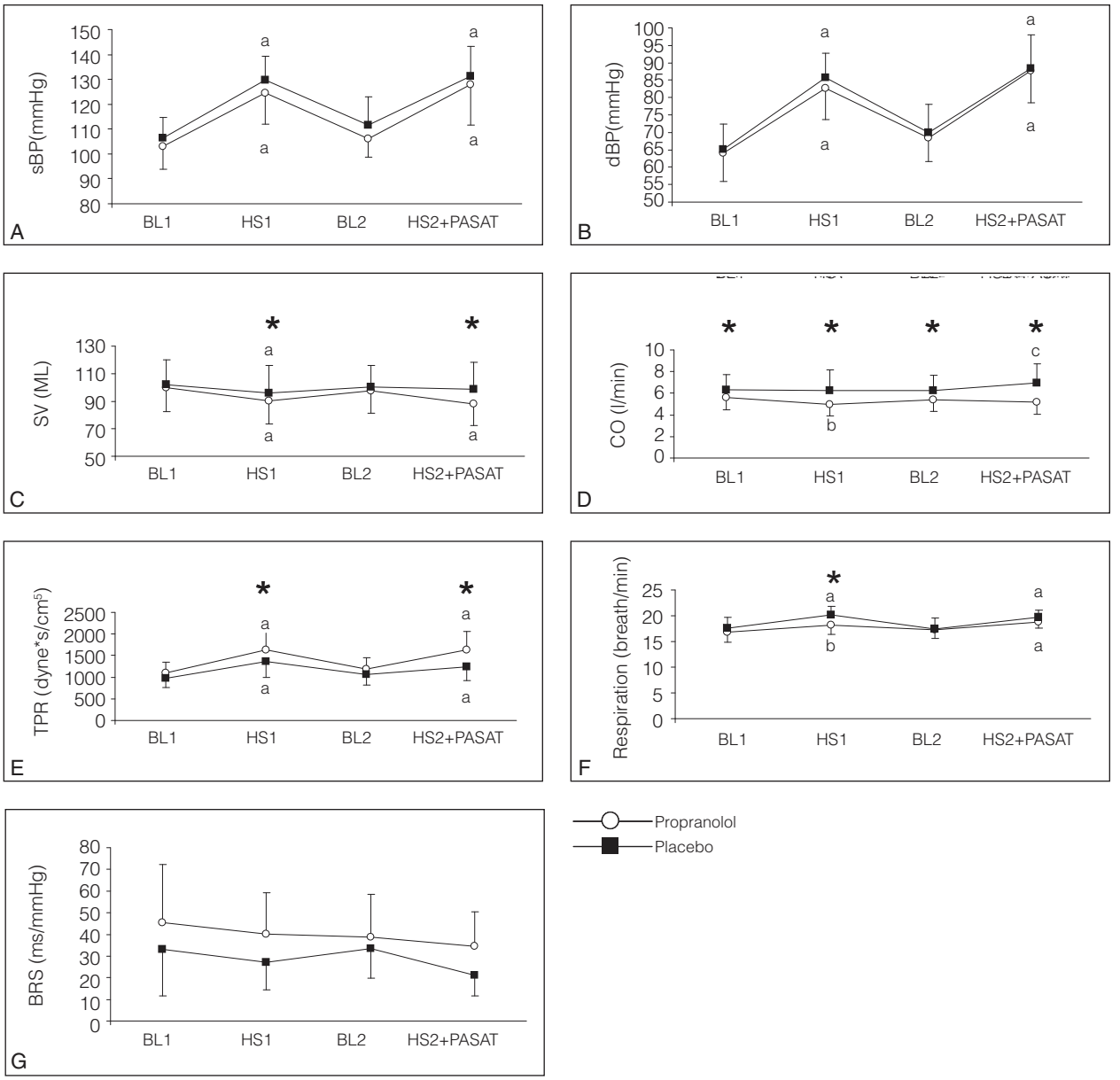

Fig 5 Hemodynamic parameters (means ± SD). (A) sBP, systolic blood pressure. (B) dBP, diastolic blood pressure. (C) SV, stroke volume. (D) CO, cardiac output. (E) TPR, total periferal resistance. (F) Respiration, breath per minute. (G) BRS, baroreceptor sensitivity. HS1 = first hypertonic saline (HS) infusion, HS2+PASAT = second HS infusion with simultaneous performance of the Paced Auditory Serial Addition Task (PASAT). BL1 and BL2 = Baseline 1 and 2. n = 16. *$P < .05$ propranolol different from placebo. Within-session differences: a = $P < .05$ different from both BL1 and BL2; b = $P < .05$ different from BL1; c = $P < .05$ different from BL1, HS1, and BL2.

propranolol produces analgesia.[12,36] However, since propranolol is an efficient anxiolytic at the dosage used in this study and no analgesic effect was observed, results from the present study do not support this theory.

The present study is, according to the authors' knowledge, the first study in which the effect of orally administered propranolol on HS-evoked pain during autonomic function monitoring has been investigated. From a patient's point of view, oral drug administration is preferred compared with other invasive routes of drug administration, considering factors like patient comfort. In clinical practice, the propranolol dosage in each patient is individual since there is a wide interindividual variation in peak plasma levels for a given dose.[37] This variability influences the therapeutic response, and the lack of effect of propranolol on perceived experimental

pain levels in this study may be due to subtherapeutic dosing. However, this study utilized an acute, nociceptive pain of peripheral origin, whereas in patients, pain may reflect peripheral and/or central (central sensitization) depending on its chronicity. The results indicate that acute, nociceptive masticatory muscle pain is probably not susceptible to propranolol, but they do not exclude the possibility that increases in central sensitization and/or reduced endogenous pain inhibition, both of which may occur in TMD pain,[18] might be affected by propranolol. In the Tchivileva et al study, the same dose was given, divided twice daily for 1 week, and this administration paradigm of 20 mg of propranolol twice a day reduced the clinical pain levels.[11]

Another explanation for the lack of effect of propranolol on HS-evoked pain in the present study might be due to a genetic variant of COMT in this group of healthy women, ie, high COMT enzymatic activity. It has been shown that a human genetic variant of COMT, encoding high COMT enzymatic activity, reduced pain sensitivity and also the risk of developing myofascial TMD.[5] If pain patients are to be treated with β-adrenergic receptor antagonists (β-blocking agents) like propranolol, it would be appropriate, prior to treatment, to identify those patients who carry the low COMT activity genetic variant and those who do not, and thereby in advance to be able to distinguish between potential responders and nonresponders to propranolol therapy. Furthermore, if β-blocking agents are to be used in the management of pain, it has to be considered that due to the effect of β-blockage, these drugs are contraindicated in pain patients who suffer from common disorders such as asthma or diabetes. Also the possible adverse effects of β-blocking agents are noteworthy, as propranolol has been shown to affect central nervous system function.[14]

Heart Rate Variability

In the present study, treatment with propranolol decreased heart rate and increased heart rate variability (SDNN and RMSSD) at all time points (rest, pain, and pain and mental arousal) compared with placebo. For that reason, treatment with propranolol during pain alone and during pain and arousal from PASAT resulted in a significantly "healthier" cardiovascular condition than treatment with placebo. From the secondary heart rate variability outcome parameters, the decrease in heart rate during propranolol treatment was probably due to an increased parasympathetic activity, which was reflected as increased HF-power, CCV-HF, HF-norm, total power, and decreased LF/HF ratio. The parasympathetic nervous system plays a central role in pain modulations, as supported by an antinociceptive effect of vagal nerve stimulation in epileptic patients[38] and inhibition of nociceptive traffic in the spinal cord in monkeys following vagal stimulation.[39] Vagal damage or vagotomy enhance bradykinin-induced mechanical hyperalgesia[40] and aggravate experimentally induced painful neuropathy in rats.[41] However, in the present acute experimental pain model, a short-term increase in parasympathetic activity during propranolol treatment had no measurable analgesic effect on pain.

Cardiovascular Responses

The sBP/dBP levels were significantly increased from baseline values during both pain alone and pain combined with PASAT. However, no differences in sBP/dBP levels between propranolol and placebo were found. Several studies have demonstrated that an increase in blood pressure in normotensive subjects is associated with a decrease in pain sensitivity (termed hypertension-related hypoalgesia), probably through baroreceptor activation.[42–44] The arterial baroreflex is the most important mechanism for short-term regulation of the arterial blood pressure, through which an increase in blood pressure activates the baroreceptors and results in a compensatory decrease in cardiovascular sympathetic activity. Conversely, a decrease in blood pressure reduces the baroreceptor activity and results in an increase in sympathetic activity and vagal inhibition.[45,46] In the present study, a low dosage of propranolol was deliberately chosen to minimize the risk of a decrease in blood pressure, which would have affected the baroreceptors and thereby paradoxically influenced the pain response. As expected, antagonism of β-adrenergic receptors by propranolol treatment resulted in a decreased SV and CO and an increased TPR compared with placebo.[47]

Methodological Considerations

The randomized, double-blinded, and placebo-controlled design is a major strength of the present study. The volunteers acted as their own controls with the use of a paired design carried out in a crossover manner. Yet, the relatively small sample size and the high numbers of statistical tests are weaknesses. In this study, only female subjects were included. This was because of the higher prevalence of myofascial TMD in women than in men[4] and the possible sex differences in the autonomic response to stress-inducing factors[48,49]; it also eliminated the risk of variability caused by sex differences in pain

perception.[50,51] However, alterations in the level of the female sex hormone estrogen during the menstrual cycle may have caused variation in muscle pain sensitivity.[52]

Another limitation is that some participants may have experienced withdrawal from nicotine or caffeine at the time of the experiment. Participants had at least 12 hours of smoking abstinence prior to an experimental session, but there may be a longer effect of smoking on the heart rate variability.[53] However, none of the participants reported being regular smokers and there were no subject-based verbal reports of stress due to smoking abstinence or any other of the requirements.

Cognitive distraction is an effective method to reduce pain.[54,55] In the present study, it is likely that distraction is one of the mechanisms by which PASAT induced analgesia. This has to be taken into consideration when interpreting the results. Also, the outcome in this study is based on acute experimental pain in a group of healthy women. Nonetheless, human experimental pain research is considered a natural link between animal research and clinical trials in chronic pain patients.[56]

Conclusions

A single dose of propranolol had no analgesic effect on acute HS-induced pain in the masticatory muscle in a group of healthy women. However, propranolol influenced the tone of the autonomic nervous system, possibly reflecting an anxiolytic effect.

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