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Noradrenaline and cortisol changes in response to low-grade cognitive stress differ in migraine and tension-type headache

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Abstract The goal of this study was to explore the relationship between indicators of sympathoneural, sympathomedullar and hypothalamic-pituitary-adrenocortical (HPA) activity and stress-induced head and shoulder–neck pain in patients with migraine or tension-type headache (TTH). We measured noradrenaline, adrenaline and cortisol levels before and after low-grade cognitive stress in 21 migraineurs, 16 TTH patients and 34 controls. The stressor lasted for 60 min and was followed by 30 min of relaxation. Migraine patients had lower noradrenaline levels in blood platelets compared to controls. Pain responses correlated negatively with noradrenaline levels, and pain recovery correlated negatively with the cortisol change in migraineurs. TTH patients maintained cortisol secretion during the cognitive stress as opposed to the normal circadian decrease seen in controls and migraineurs. There may therefore be abnormal activation of the HPA axis in patients with TTH when coping with mental stress, but no association was found between pain and cortisol. A relationship between HPA activity and stress in TTH patients has to our knowledge not been reported before. In migraine, on the other hand, both sympathoneural activation and HPA activation seem to be linked to stress-induced muscle pain and recovery from pain respectively. The present study suggests that migraineurs and TTH patients cope differently with low-grade cognitive stress.

Keywords Catecholamines • Cortisol • Migraine • Tension-type headache • Stress

Introduction

Stress may trigger headache in both migraine and tension-type headache (TTH) patients [1–4]. An abnormal and prolonged stress response has been hypothesised to cause chronic pain [5, 6], and pain processing seems to be abnormal in TTH [7–9] and in migraine [10, 11]. Stress increases sympathoneural and sympathomedullar sympathetic nervous system activity and it may also activate the hypothalamic-pituitary-adrenocortical (HPA) axis, but it is not known if this activation is correlated to pain and headache development during a stressful task. Noradrenaline in blood plasma is considered an indicator of sympathoneural activity, as most of the circulating NA is released from sympathetic nerve endings, particularly in muscle [12]. Plasma adrenaline is released mainly by the adrenal medulla and reflects part of the subject's sympathomedullar activity. The primary marker for HPA activity in humans is cortisol, having complex and diverse effects throughout the body [13, 14].

Sympathetic activity has been measured by means of biochemical markers in both migraineurs [15–18] and TTH patients [16, 19]. Some of these studies investigated biochemical effects of short-lasting stress from stressors such as cold pressor tests, tilt tests, mental arithmetic tests etc. It may be argued that these short-lasting stressors are of limited relevance with respect to long-lasting, low-grade stressors often reported to induce headaches in daily life. In the present model [20–22], we sought to create a low-grade cognitive stressor in order to simulate real-life stress in an office environment.

Elevated plasma cortisol has been reported in migraine [23, 24] and a trend towards higher cortisol has been reported in TTH [23]. The cortisol response to low-grade cognitive stress has to our knowledge not been studied in headache patients previously. In this paper, we report cortisol, noradrenaline (NA) and adrenaline changes in response to stress in patients and controls, and consider potential correlations between the biochemical variables and pain responses and pain recovery. We have also considered the possibility that HPA activation (cortisol secretion) might be correlated to cardiovascular reactivity in migraine and TTH.

Materials and methods

Subjects

The background data of all the subjects that entered the physiological study have been published previously [20]. Background data on subjects with biochemical data are displayed in Table 1 (due to technical problems, 10 controls, one migraineur and two TTH patients, had no biochemical data). There were no differences between the patients and controls for these data. Patients were diagnosed according to the International Headache Society classification of headache from 1988 [25]. Control subjects did not suffer from headache or musculoskeletal pain for more than one day per month. Exclusion criteria were: neoplastic disease, hypertension, infectious disease, metabolic, endocrine or neuromuscular diseases, significant psychiatric disorders, connective tissue disorder, tendinitis, recent significant accident or injury, pregnancy, daily medication with neuroleptics, antiepileptics, Ca^{2+}-blockers, β-blockers, antidepressants and significant associated diseases affecting either the heart, lungs, cerebrovascular system, central or peripheral nervous system. Migraineurs with TTH more than 7 days per month were also excluded. The migraine patients were recruited from our tertiary headache facility. Patients referred to such a facility often have more severe headaches or more complex symptoms (such as auras), and among these patients the proportion of women is higher than would be expected from the gender ratio of migraineurs in the general population. The project was approved by the Regional Ethics Committee and performed in accordance with the 1964 Declaration of Helsinki. All participants gave written informed consent. The participants were provided with written information concerning the aim of the study prior to the day of the stress test. The aim of studying pain and headache was mentioned, but the information focused on the practical details of the procedure.

Questionnaire and interview

Patients arrived at our facility around 8 a.m. and underwent a structured interview concerning headaches and musculoskeletal complaints (distribution, severity and duration) prior to the stress test. The interview was conducted in a calm and relaxed manner with the subject sitting comfortably, and lasted around 30 min. One of the interview questions was: "Please state the level of general tension you have felt during the last 2–3 months", and the response was scored on a visual analogue scale (VAS) with endpoints: not tense–very tense. Participants also kept a headache diary for 7 days before and after the stress test. Twelve of 21 migraineurs reported a migraine attack within two days before the stress test, while 11 patients reported an attack within two days after the stress test. The neuroticism index of the Eyseneck Personality Questionnaire (EPQ-N) scores was also calculated from the questionnaire (Table 1). Two questions in this questionnaire dealt with symptoms of depression, and there were no group differences in the answers to these questions. At the end of the interview, the first blood sample was drawn by venipuncture.

Procedure

The stress test procedure is described in detail in another paper [20], and only a short summary will be given here. The subjects performed a two-choice reaction-time test presented on a PC monitor for 60 min. They were instructed to perform the test as quickly and cor-

rectly as possible, and were provided with feedback on their performance throughout the test. They were also informed that they would be monitored through a video camera. The subjects were acclimatised to the laboratory environment for 30 min, during which the procedure was explained and the recording electrodes were attached to the patient. The technician told the subject to relax and then left the room. The recording started with 5 min uninstructed rest (UIR), followed by 5 min active, instructed rest with visual EMG feedback (FB). During the FB period the technician instructed the subject in a calm, quiet manner on how to relax the muscles more efficiently based on the patient's own EMG data shown on the computer screen. The cognitive task was then performed for 1 h (800–1500 trials), followed by 30 min recording during rest (recovery period). The subjects were asked to relax while seated and to move as little as possible during the recovery period. After the UIR and FB periods, at 10-min intervals during the cognitive task, and at 10-min intervals during the recovery period, the subjects were asked to mark on a VAS scale their level of pain (no pain–worst bearable pain). Pain was reported bilaterally for the forehead, temples, neck and shoulder (upper trapezius area). No patient had to be excluded because of headache attacks during the test. EMG was recorded bilaterally at the forehead, temples, neck and upper trapezius. Systolic blood pressure (BP_{sys}), diastolic blood pressure (BP_{dia}), heart rate (HR) and finger blood flow (BF) were continuously recorded during the test, and mean values calculated for the UIR and FB period, and for each 10-min interval throughout the stress test and recovery period.

Venous blood was sampled again immediately after the stressful task. The two blood samples were therefore taken with an interval of 2–3 h. More frequent sampling by a cannula was considered but rejected because we anticipated that it would interfere with pain, tension, EMG and cardiovascular measurements. Patients were able to relax before the first blood sample, and both the nurse and the technician involved in the stress test were instructed to act in a quiet, calm manner at all times. The stress test was performed in a quiet room with no distractions. So while we were unable to control for stressful events that the subjects might have experienced prior to arriving at our facility, we were careful to avoid unnecessary stressful situations after arrival.

Biochemical analyses

Blood was collected into EDTA vacutainers and immediately placed in ice water or into vacutainers without an anti-coagulant. Non-coagulated blood was centrifuged for 10 min at 300 g (at a temperature of 4°C) to obtain platelet-rich plasma (PRP). After withdrawing an adequate sample of PRP for catecholamine analysis and platelet counting, samples were centrifuged again for 10 min at 3000 g (4°C) to obtain platelet-poor plasma (PPP). Serum was collected after 30 min coagulation, by centrifugation at 1500 g, 10 min, at room temperature. All samples were stored at –80°C prior to analysis. Plasma catecholamines were extracted by adsorption to aluminium oxide [26] and analysed by HPLC (Merck Hitachi LaChrom system, Darmstadt, Germany) with electrochemical detection. Catecholamines were separated on a LiChroCART 250-4 column containing LiChrospher 100 RP-18 (5 µm) (Merck, Darmstadt, Germany), using a sodium acetate buffer (pH 4.8) and methanol (8.5 vol%) as eluents [27]. External standards were used for calculation of sample catecholamine concentrations. Cortisol concentrations in serum samples were determined using a competitive enzyme immunoassay kit (R&D Systems, Abingdon, UK). Serum samples were diluted 8-fold, processed and analysed by absorbance reading (Titertek Multiscan, Titertek, AL, USA) at 405 nm, according to the manufacturer's procedure. Catecholamine

Table 1 Background data on subjects included in the study

Diagnostic group	Controls (n=34)	Migraine (n=21)	Tension-type headache (n=16)
Gender ratio (F:M)	30:4	19:2	7:9
Mean age (range)	41.0 (19–61)	41.2 (21–60)	35.6 (19–52)
Mean number of years with headache (range)	–	20.1 (7–37)	8.7 (0–32)
Number of subjects with chronic headache (%)	–	4 (19.0)	12 (75.0)
Mean duration (h) of headache attacks (range)[a]	–	30 (1–72)	–
Number of subjects with aura (%)	–	12 (57.1)	–
Mean general tension (VAS) (range)	29.7 (0–84)	36.0 (1–87)	26.5 (0–65)
Mean EPQ-N score (SD)	7.4 (4.2)	9.0 (4.0)	8.25 (4.9)
Number of subjects who smoke (%)	10 (29.4)	6 (28.6)	2 (12.5)
Body mass index (SD)	25.1 (3.6)	24.0 (3.3)	25.1 (4.5)
Days since last menstruation (SD)[b]	17.0 (11.9)	19.2 (14.8)	17.1 (17.2)

[a]One migraine patient had some attacks of short duration

[b]Fourteen women (7 controls, 6 migraineurs and 1 TTH patient) had started menopause. Three women had for unknown reasons reported more than 35 days since their last menstruation (1 control, 1 migraineur and 1 TTH patient)

analyses were done for 27 controls, 19 migraineurs and 14 TTH patients. Cortisol analyses were done for 24 controls, 17 migraineurs and 13 TTH patients. Adrenaline levels in PRP suggested degradation, probably by MAO-B [28], and are not shown.

Data analyses

Levels of plasma NA and adrenaline, platelet NA and cortisol, as well as plasma platelet levels (not shown) were calculated before and after the stress test. Biochemical changes were calculated from the difference in concentration after the test compared to levels before the test.

The pain variables used in the statistical analyses were pain response and pain recovery. The pain response was defined as the highest pain response (max pain at $t_{10–60min}$–pain at t_{0min}) among the 8 location- and side-specific responses. The minimal pain during recovery was used first to calculate 8 location- and side-specific pain recoveries (minimal pain at $t_{75–95min}$–pain at t_{0min}). Thereafter, the highest among these 8 location- and side-specific pain recoveries was defined as pain recovery. Mean cardiovascular responses (mean level $t_{(0–10min)–(50–60min)}$–baseline) and mean cardiovascular recovery (mean level $t_{(65–75min)–(85–95min)}$–baseline) were used in analyses involving BP_{sys}, BP_{dia} or HR.

Based on the goal of the study, the following variables were used in correlation analyses: (a) biochemical changes *vs*. pain response and recovery, (b) pre-test biochemical levels *vs*. pain response (c) post-test biochemical levels *vs*. pain recovery, (d) post-test biochemical levels *vs*. pain response, (e) cortisol change *vs*. mean cardiovascular responses, (f) pre-test cortisol level *vs*. mean cardiovascular responses and (g) post-test cortisol level *vs*. mean cardiovascular recovery. Correlations between catecholamine changes and cardiovascular responses were not included in the study aim and hence not reported.

A few subjects had partly missing data due to technical difficulties: two controls and two migraineurs had corrupted BP and HR data during the test and recovery period. One control had missing pain data at t_{95min}, while one patient with TTH had corrupted BP, HR, BF and pain data during the recovery period.

Statistics

Kolmogorov-Smirnov's test was used to confirm that all (ln-transformed) biochemical data were close to a Gaussian distribution. Differences in biochemical levels pre- and post-test were analysed by paired Student's *t*-test. Analysis of variance (ANOVA) with repeated measures was used for intergroup comparisons of biochemical changes in response to stress (time×group interactions). One-way ANOVA with post-hoc Tukey's test was used for intergroup comparisons of pre-test variables. For pain data the non-parametric Spearman's rank order ρ was used for correlation analysis. Pain responses were power-transformed by a factor $x^{0.5}$ for a closer Gaussian distribution when used as a covariate in ANOVA analyses. A two-tailed significance level of <0.05 was considered significant. *P*-values within a range of 0.05–0.10 were considered as trends.

Results

Pre-test, post-test and response means are shown in Table 2. Pre- and post-test levels of plasma NA and adrenaline, and platelet NA and cortisol are shown in Figure 1.

Biochemical changes in response to stress

We found no significant differences in pre-test biochemical levels between any of the diagnostic groups. Significant differences in pre- *vs*. post-test levels were found for cortisol in controls (p″0.005) and migraineurs (p=0.008) but not in TTH (p=0.93). A trend (post-hoc Tukey's, C *vs*. M, p=0.07) towards lower pre-test platelet NA was found in migraine patients compared to controls (Table 2). Catecholamine mean levels changed little, but tended to be lower after the test in controls and migraineurs, and remain stable or be higher after the test in TTH.

To compare biochemical levels and biochemical changes between the three groups, two-group ANOVA analyses were done (Table 3). Platelet NA levels were found to be significantly lower in migraineurs compared to controls, and plasma NA levels in migraineurs tended to be lower than in controls. TTH patients had a significantly different cortisol change (no decrease with time) compared to controls or migraineurs.

Correlations between pain and biochemical variables

Pain development is shown in Figure 2, and Table 4 shows data on the pain variables used in the correlation analyses. Migraineurs had negative correlations between pre-test platelet NA and pain responses (r_s=–0.55, p=0.02), and between post-test plasma NA and pain responses (r_s=–0.58, p=0.009). Migraineurs also showed a negative correlation between pain recovery and the cortisol response (r_s=–0.52, p=0.03). TTH patients had a negative correlation between pain recovery and the plasma NA response (r_s=–0.64, p=0.01). There were no correlations between biochemical variables and pain in controls.

Correlations between cardiovascular variables and cortisol

Figure 3 shows the cardiovascular development during and after the stress test, and Table 4 shows the cardiovascular variables used in the correlation analyses. In controls, the

cortisol change was positively correlated to the BP_{sys} responses (r_s=0.55, p=0.01). The pre-test cortisol was also correlated positively with the mean BF responses in controls (r_s=0.44, p=0.03). In TTH patients pre-test cortisol correlated negatively with the HR mean response (r_s=–0.57, p=0.04), and there was a trend towards a positive correlation between the cortisol change and the mean HR response (r_s=0.49, p=0.09). In migraineurs pre-test cortisol correlated positively with the mean BP_{sys} response (r_s=0.52, p=0.048).

Discussion

There is conflicting evidence on the basal levels of catecholamines in migraineurs, as both higher, equal and lower levels compared to controls have been found [17, 18, 29–31]. For patients with TTH, previous studies have demonstrated lower levels of catecholamines compared to controls [16, 19, 32]. Mean basal levels of catecholamines tended to be lower for both the migraineurs and TTH patients compared to healthy controls in our study, but the differences did not reach statistical significance.

There were no significant differences between pre- and post-test catecholamine levels in the three subject groups. Similarly, no rise in NA and adrenaline has been found after mental arithmetic tests in healthy subjects [33]. However, there were marked heart rate and BP rises during the stress in our study, indicating that sympathetic autonomic activation does occur. This may suggest that NA and adrenaline levels in the forearm are less sensitive indicators of sympathoneural and sympathomedullary activation to low-grade cognitive stress than physiological variables.

We have previously reported higher pain responses during stress in migraineurs compared to controls [20]. The present paper shows that platelet and plasma NA levels were lower in migraineurs than in controls, suggesting that migraineurs may have noradrenergic hypofunction, potentially related to the

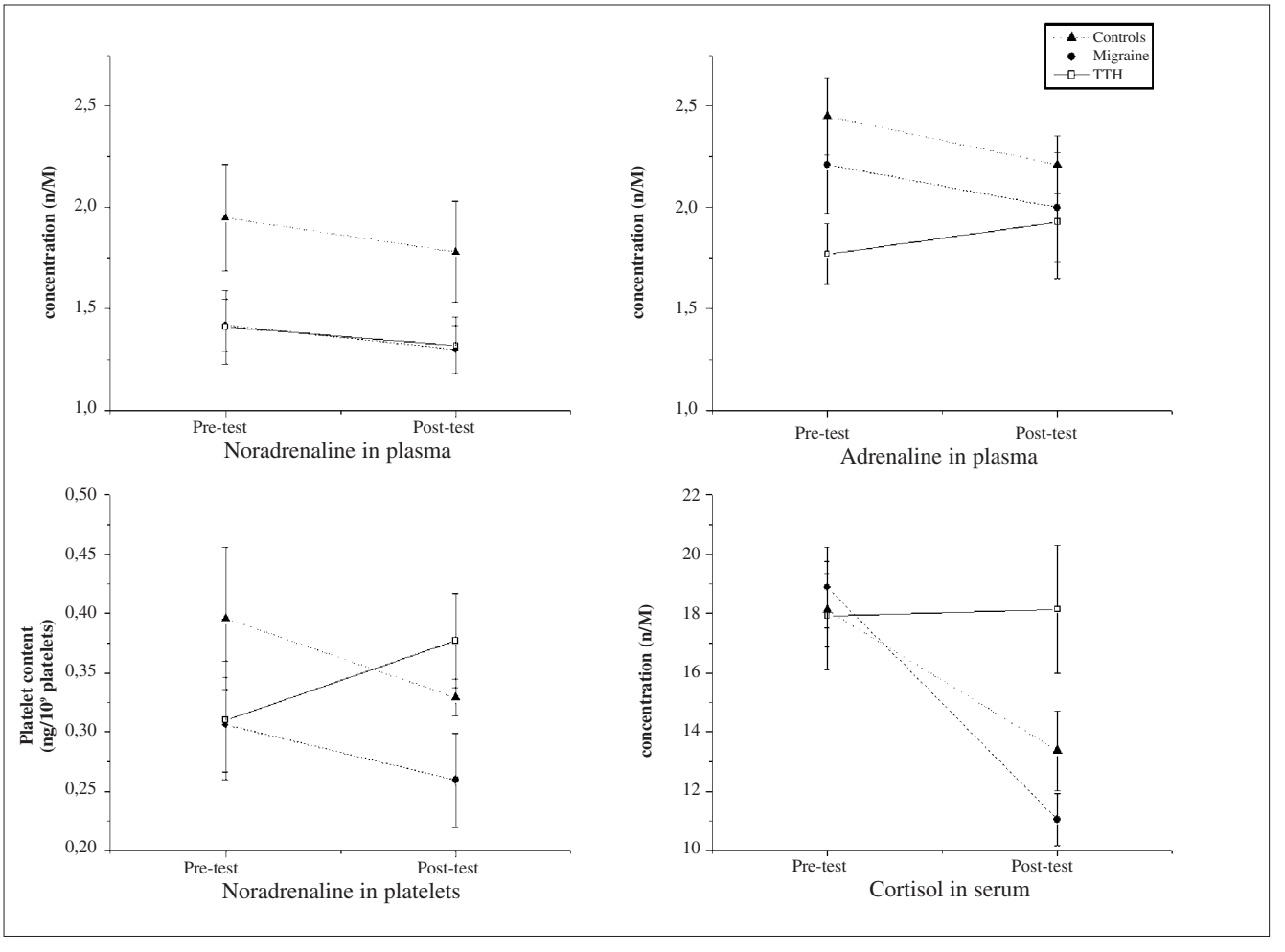

Fig. 1 Pre- and post-test mean values (SEM) of noradrenaline in plasma, noradrenaline in platelets, adrenaline in plasma, and cortisol in healthy controls and patients with migraine or TTH

mechanism for stress-induced pain. The inverse relationship between pain responses and both pre-test platelet NA and post-test plasma NA indicates that stress-induced pain increases at lower levels of NA. Altered central noradrenergic activity has previously been indicated in patients with chronic migraine, as evidenced by an inverse correlation between clonidine-induced β-endorphine secretion and pre-test pain [34].

TTH patients have delayed pain recovery after stress compared to controls [20]. In the present study, TTH patients showed a negative correlation between pain recovery and the plasma NA response. Hence, it seems that low sympathoneural activation is associated with prolonged pain during recovery in TTH-patients. A blunted acute BP and HR response to stress onset is compatible with low sympathoneural activation in TTH.

Cortisol levels in TTH patients did not change significantly during the test, in contrast to the reductions in controls and migraineurs. During a normal circadian rhythm cortisol levels are high in the morning and decrease throughout the day [35]. The test subjects arrived at our facility between 08:00 and 08:45 and had their pre-test blood sample taken 30 min later. As the post-test samples were not taken for another 2.5–3 h, a decrease in cortisol levels could be expected. As such a decrease was found in both controls and migraineurs, the test did not appear to affect the normal rhythm. However,

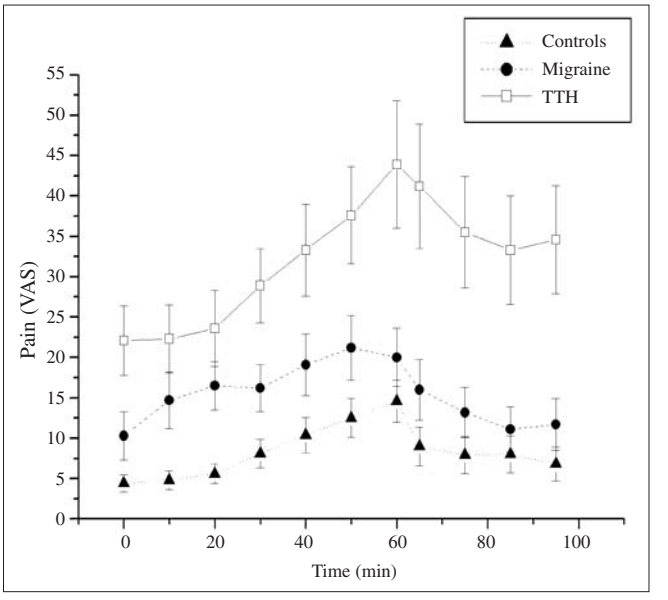

Fig. 2 Pain development throughout the stress test and recovery period. Values are given as group means (SEM), where maximal reported pain (from the trapezius, splenius, temporalis and frontalis areas, irrespective of side) for each subject was used in the calculations. 0–60 min, duration of the cognitive stress test. 65–95 min, relaxation period after the test

Table 2 Pre- and post-test levels and response mean values (SD) for noradrenaline in plasma, noradrenaline in platelets, adrenaline in plasma, and cortisol in controls, migraineurs and TTH patients

		Controls (n=34)*	Migraine (n=21)*	Tension-type headache (n=16)*
Noradrenaline	Pre-test	1.95 (1.36)	1.42 (0.56)	1.41 (0.66)
in plasma (nM)	Post-test	1.78 (1.26)	1.30 (0.51)	1.32 (0.53)
		–0.21 (0.51)	–0.12 (0.47)	–0.09 (0.31)
Noradrenaline	Pre-test	0.40 (0.30)	0.31 (0.16)	0.31 (0.18)
in platelets	Post-test	0.33 (0.43)	0.26 (0.15)	0.38 (0.16)
(ng/10^9 platelets)		–0.07 (0.47)	–0.05 (0.21)	0.07 (0.25)
Adrenaline	Pre-test	2.45 (0.94)	2.21 (1.02)	1.77 (0.55)
in plasma (nM)	Post-test	2.21 (0.69)	2.00 (1.17)	1.93 (1.06)
		–0.26 (0.84)	–0.17 (0.70)	0.16 (0.85)
Cortisol	Pre-test	18.9 (6.6)	18.1 (5.1)	17.9 (6.58)
in serum (nM)	Post-test	11.1 (4.3)	13.4 (5.5)	18.1 (7.8)
		–7.84 (7.76)	–4.75 (7.68)	0.22 (6.44)

Response = difference between post-test and pre-test levels

*N shown for patients with either catecholamine or cortisol analysis (or both). Specific n for each analysis: catecholamines: C=27, M=18, TTH=14; cortisol: C=24, M=17, TTH=13

Trend towards difference between patients and controls (ANOVA w/post-hoc Tukey's test, 0,05 $p<0.1$)

Significant difference in intragroup pre- and post-tests levels (paires Student's t-test, $p<0.05$)

the lack of cortisol change during the test in TTH patients suggests either that they have increased HPA activity during stress and thus maintain their cortisol levels despite the natural circadian decrease, or perhaps that TTH patients, like depressed patients [36], have a blunted circadian cortisol rhythm.

TTH patients had delayed HR and BP responses and prolonged vasoconstriction, presumably reflecting a dysfunctional sympathetic response. However, as blood was sampled only twice, it was impossible to detect any biochemical changes during the early phase of the stress period. Leone and co-workers reported a trend towards higher cortisol baseline values in migraineurs and TTH patients compared to controls [23], while Peres and co-workers found higher 24-h cortisol concentrations in chronic migraine compared to a control group [24].

The relationship between cortisol changes and pain was different in TTH and migraine. In migraineurs a higher cortisol change seemed to "protect" against delayed pain recovery, suggested by the inverse relationship between the cortisol response and pain recovery. However, there was no similar association in TTH patients. The (relatively) increased cortisol excretion during stress in TTH did not affect stress-induced pain responses or pain recovery.

Lack of HR adaptation was seen in TTH patients during stress in a previous study (submitted results), with a small initial increase in HR followed by a slowly increasing response for the remainder of the stress test. This was in contrast to controls and migraineurs, who had a larger initial

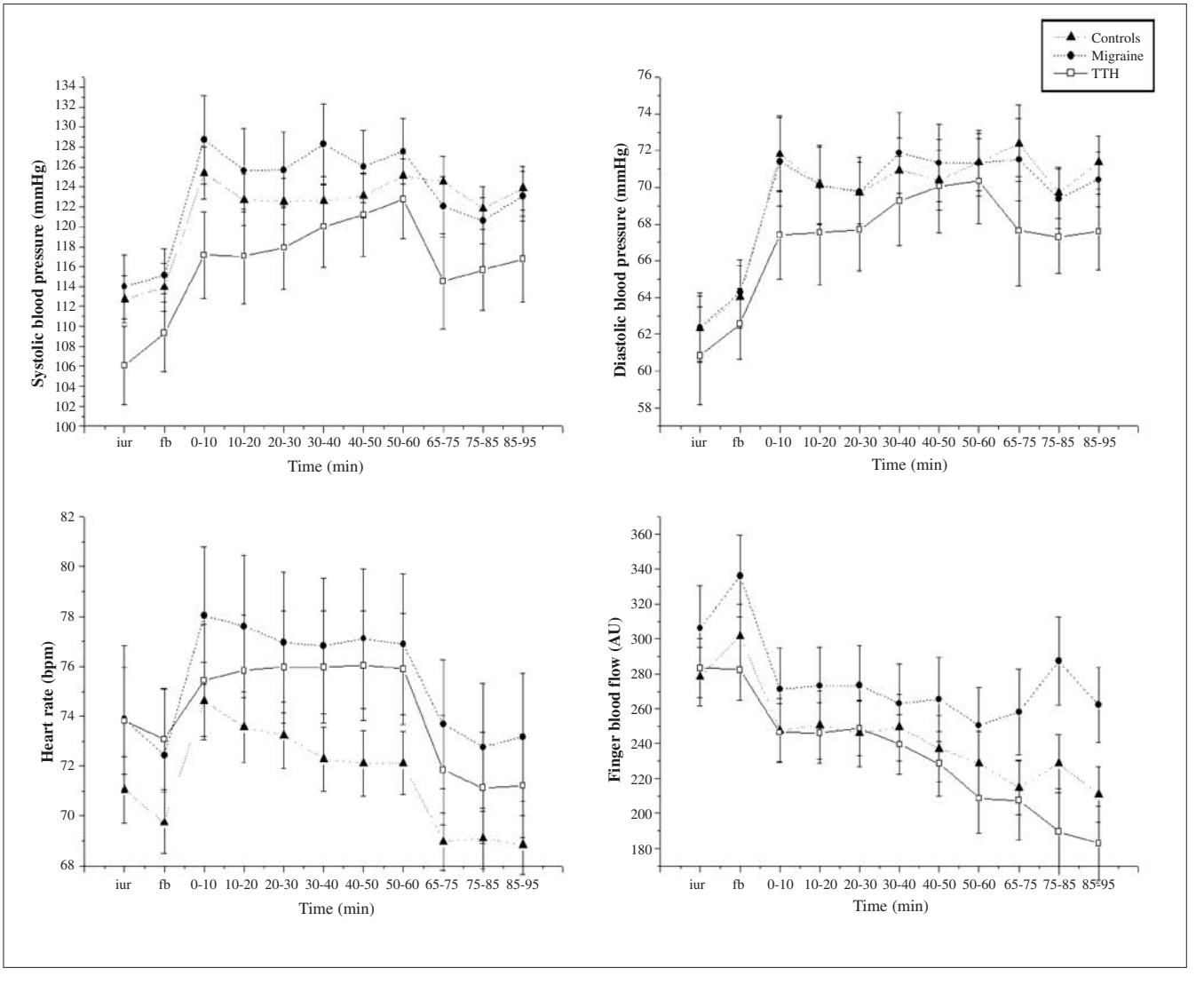

Fig. 3 Development of systolic BP, diastolic BP, heart rate and finger blood flow throughout the stress test and recovery period. Values are given as group means (SEM). UIR, uninstructed rest period (baseline EMG). FB, EMG feedback aided rest period. 0–60 min, duration of the cognitive stress test. 65–95 min, relaxation period after the test

increase followed by a decline in HR throughout the test. In the present paper we show that cortisol levels were maintained during the stress test in TTH patients. Cortisol can enhance vascular reactivity, for instance by increasing the effects of circulating NA and adrenaline, and it is therefore likely to affect HR development during the test. There was a trend towards a positive correlation between the cortisol response and the mean HR response, which is in accordance with the predicted effect of cortisol secretion.

There are studies indicating that the way subjects perceive and cope with a stressor influences HPA activity. A meta-analysis showed that performance tasks perceived as social-evaluative, especially those with uncontrollable factors, provoked a greater release of cortisol than tasks without such factors [37]. Our stress model involved such social-evaluative aspects, as the subjects knew they were being monitored through a video camera. There were also uncontrollable factors, as the patients were instructed to sit still and work as fast and correctly as possible, with no way to take breaks or move to relieve themselves from pain or stress. Hence, the fact that TTH patients in our study maintained their cortisol levels during the stressful task suggests that they cope differently with the stressor than controls or migraineurs.

The methodology for sampling cortisol from our subjects may have been sub-optimal. Dickerson and Kemeny [37] suggest that cortisol assessments 21–40 min from stressor onset should be obtained in order to detect the peaks in cortisol levels, while our results were sampled 60 min after

Table 3 Test statistics for repeated measures two-group ANOVA analyses

		Time×group interaction*		Group effect*	
Noradrenaline in plasma	C *vs.* M	$F(42,1)=0.002$	$p=0.96$	$F(42,1)=3.46$	$p=0.07$
	C *vs.* TTH	$F(37,1)=0.14$	$p=0.72$	$F(37,1)=0.76$	$p=0.39$
	M *vs.* TTH	$F(30,1)=0.09$	$p=0.77$	$F(30,1)=0.53$	$p=0.47$
Noradrenaline in platelets	C *vs.* M	$F(39,1)=0.02$	$p=0.90$	$F(39,1)=8.68$	$p=0.005$
	C *vs.* TTH	$F(36,1)=0.75$	$p=0.39$	$F(36,1)=0.006$	$p=0.94$
	M *vs.* TTH	$F(28,1)=0.12$	$p=0.73$	$F(28,1)=2.29$	$p=0.14$
Adrenaline in plasma	C *vs.* M	$F(39,1)=2.79$	$p=0.6$	$F(39,1)=1.11$	$p=0.30$
	C *vs.* TTH	$F(35,1)=0.95$	$p=0.34$	$F(35,1)=2.4$	$p=0.13$
	M *vs.* TTH	$F(29,1)=2.23$	$p=0.15$	$F(29,1)=0.06$	$p=0.81$
Cortisol in serum	C *vs.* M	$F(38,1)=0.95$	$p=0.34$	$F(38,1)=0.76$	$p=0.39$
	C *vs.* TTH	$F(34,1)=12,6$	$p=0.001$	$F(34,1)=4.86$	$p=0.03$
	M *vs.* TTH	$F(27,1)=5.09$	$p=0.032$	$F(27,1)=0.93$	$p=0.34$

*Time×group interactions reflect differences in biochemical development (changes pre- to post-test), while group effects reflect overall differences in biochemical levels between the groups
Significant difference between subject groups, $p<0.05$
Trend towards difference between subject groups, $0.05\leq p<0.1$

Table 4 Physiological/pain responses and recovery for controls, migraineurs and TTH patients

Variable		Controls (n=34)	Migraine (n=21)	Tension-type headache (n=16)
Systolic BP (mmHg)	Mean response	12.1 (8.1)	13.4 (8.5)	13.5 (10.0)
	Mean recovery	11.0 (10.0)	7.7 (13.0)	10.1 (12.9)
Diastolic BP (mmHg)	Mean response	8.7 (5.2)	8.7 (7.3)	8.1 (6.5)
	Mean recovery	8.7 (6.1)	8.1 (6.8)	7.2 (7.5)
Heart rate (bpm)	Mean response	2.2 (3.9)	3.2 (4.7)	1.5 (2.8)
	Mean recovery	–1.5 (3.7)	–0.9 (3.9)	–2.5 (4.1)
Blood flow (AU)	Mean response	–39.9 (66.1)	–39.9 (74.2)	–34.1 (46.0)
	Mean recovery	–73.1 (97.0)	–31.2 (95.7)	–80.3 (69.9)
Pain (VAS)	Max. response	15.3 (16.0)	22.6 (18.6)	36.1 (26.8)
	Max. recovery	2.9 (6.7)	4.4 (6.4)	13.6 (16.5)

Arbitrary units. Only subjects with valid biochemical data

the onset of the stressor. Additionally, some aspects of the cortisol response to stress may have been hidden as the samples were taken during the morning hours when the cortisol level was decreasing due to the circadian rhythm. However, as the test was performed in the morning for all subjects, we find it likely that the reported group differences between TTH patients and controls and migraineurs are real.

It is a weakness of our study that the sample size was limited and gender distribution in the three groups was uneven. We had some problems with recruiting enough patients and controls, resulting in groups that were smaller than intended. Technical difficulties with some of the blood samples decreased the sample size even more. The low group size and uneven gender distribution made gender-specific statistical analyses less meaningful due to a low sample power, and we have therefore based our analyses on groups consisting of both men and women. When investigating differences in biochemical changes in men and women irrespective of diagnosis (48 women *vs*. 11 men for catecholamines, 42 women *vs*. 12 men for cortisol), no gender differences could be found (results not shown). Reproductive steroid levels may also influence the stress response [38], and future work in this area is warranted. However, as female reproductive steroid levels change throughout the menstrual cycle, a large number of women would need to be recruited.

In conclusion, the two patient groups responded differently to the stressor, both compared to each other and to controls. For migraineurs the NA levels were lower than for controls, and negatively correlated to the pain response, which may indicate that the sympathoneural responses to stress protect against stress-induced pain. TTH patients on the other hand seem to have a derangement of the HPA axis. Increased HPA axis reaction to low-grade evaluative and uncontrollable cognitive stress in TTH is plausible, but the association between HPA activation and acute pain is uncertain. Such a relationship between HPA activity and stress in TTH patients has to our knowledge not been reported before. A detailed study of cortisol secretion and regulation in TTH is warranted, and more generally, further studies on the relation between headache, stress and the different biochemical and physiological stress markers may give important insights into headache pathogenesis.

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