Neuroendocrine Responses to Psychological Stress in Patients with Myofascial Pain

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Aims: *To investigate the responses of the hypothalamus-pituitary-adrenocortical and sympathetic-adrenal-medullary systems to experimentally induced psychological stress in patients with myofascial pain.* ***Methods:*** *To characterize the features of these systems, temporal variations in plasma cortisol, adrenaline, and noradrenaline concentrations in response to psychological stress were measured in 20 patients with myofascial pain and in 20 healthy controls.* ***Results:*** *The concentrations of plasma cortisol, adrenaline, and noradrenaline in response to psychological stress were significantly higher in the pain patients than in the healthy controls. Furthermore, although the plasma cortisol, adrenaline, and noradrenaline concentrations were significantly increased from the basal levels in both groups, the rate of recovery from these levels was significantly slower in patients than in healthy controls.* ***Conclusion:*** *These results suggest that both the sympathetic-adrenal-medullary and hypothalamic-pituitary-adrenocortical systems are more highly activated in response to psychological stress in patients with myofascial pain than in healthy individuals.*
J OROFAC PAIN 2005;19:202–208

Key words: myofascial pain, psychological stress, cortisol, adrenaline, noradrenaline

One of the most frequently suggested mechanisms causing myofascial pain associated with temporomandibular disorders (TMD) is hyperactivity of the masticatory muscles.[1–3] Stress-related increases in muscle tension have especially been implicated in the development of myofascial pain. Imagining a stressful situation resulted in more electromyographic activity in patients with myofascial pain than in healthy controls,[4] in both the frontalis muscle and masseter muscles.[5] Furthermore, several studies have shown associations between myofascial pain and psychological factors in TMD patients,[6–8] although it is not clear whether these psychological factors are the cause or the result of certain painful conditions.[9]

Psychological stress is known to induce various adaptational responses of physiologic systems, including increased activity in the hypothalamic-pituitary-adrenocortical (HPA) system, which promotes cortisol secretion from the adrenal cortex, and increased activity in the sympathetic-adrenal-medullary (SAM) system, which secretes adrenaline and noradrenaline through peripheral sympathetic nerve endings and the adrenal medulla.[10–12] Kirschbaum et al reported in detail on the reaction of these 2 systems to psychological stress in terms of gender differences, individual differences, the effect of estradiol treatment, personality factors, mood, age, menstrual cycle phase, resilience of these systems,

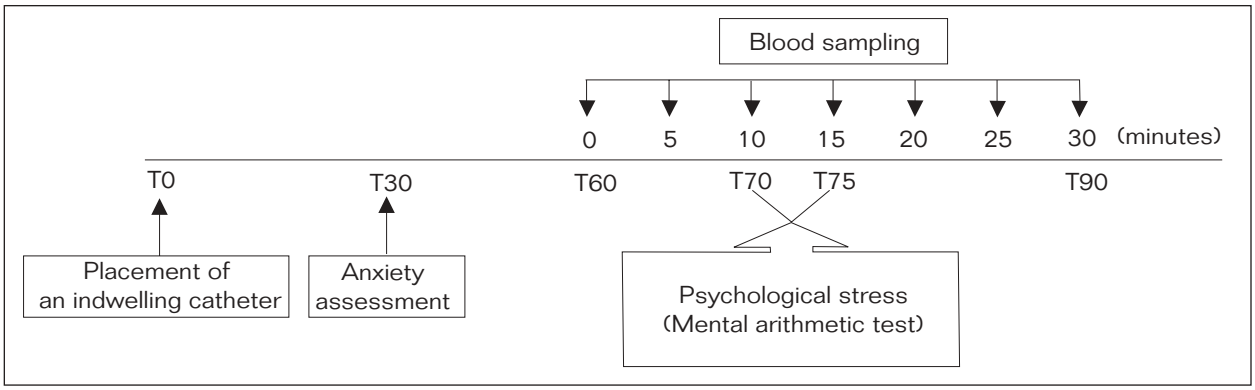

Fig 1 Experimental protocol.

the rate of post-stress recovery of these systems, the effect of suckling, the effect of glucose, and habituation of these systems.[12–23]

Studies over the past decade have shown an association between hyperactivity of the HPA and SAM systems and anxiety disorders in psychological response to stress.[24,25] Therefore, it was conjectured that the responses of these 2 systems to psychological stress differ between patients with myofascial pain and healthy controls. Up to now, temporal variations in plasma cortisol, adrenaline, and noradrenaline concentrations in patients with myofascial pain have not been determined in relation to anxiety levels. In the present study, the responses of these 2 systems to experimentally induced psychological stress were examined, and the features of these systems in patients with myofascial pain were characterized.

Materials and Methods

Subjects

Twenty female patients (mean age ± SD, 21.55 ± 1.96 years old) participated in this study. All of them attended Kagoshima University Dental Hospital and were diagnosed with myofascial pain. Criteria for the diagnosis of myofascial pain included *(1)* unilateral or bilateral tenderness to palpation in the masseter, temporalis, digastric, medial pterygoid, sternocleidomastoid, splenius capitis, or trapezius muscles; *(2)* report of pain at maximal mouth opening; *(3)* no internal derangement of either temporomandibular joint (TMJ) as judged by auscultation; *(4)* no reciprocal clicking sounds during a clinical examination that included vertical and horizontal function of the mandible; and *(5)* no other painful condition as the primary diagnosis.[5] Patients with myofascial pain were excluded if they were wearing any intraoral appliance or taking any muscle-relaxing medication. Twenty female volunteers (20.30 ± 1.13 years old) also participated as healthy controls. None of the controls had a history of myofascial pain symptoms or other TMJ dysfunction. All of the patients and controls were college students and appeared to have similar educational levels. All of them provided written informed consent to participate in the study, which was approved by the Ethics Committee of the School of Dentistry, Kagoshima University.

Experimental Protocol

All women were tested during the follicular phase of their menstrual cycle. They were instructed to refrain from eating or drinking for at least 90 minutes before the start of the experiment. Blood sampling, measurement of anxiety level, and a mental arithmetic test were performed in a quiet, isolated room that was maintained at 23 ± 1°C and 60% ± 5% humidity. Only 1 participant and 1 experimenter were in the room.

Blood Sampling. An indwelling catheter was placed in a vein in the forearm of each participant at 1300 hours (T0). The same experimenter subsequently collected blood (2 mL) into clotted serum tubes for cortisol analysis, and 4 mL was collected into tubes containing EDTA for adrenaline and noradrenaline analyses at 5-minute intervals from 60 minutes after the experiment (T60) to T90 (Fig 1). Plasma was immediately separated by centrifugation (+4°C, 2,500 *g*, 15 minutes) and stored at –40°C.

Anxiety Level. Thirty minutes after T0 (ie, at T30), each participant was asked to answer the Spielberger State-Trait Anxiety Inventory (STAI)[26] to estimate anxiety levels. This scale consists of 2 subscales, each with 20 statements that measure state and trait anxiety. The score for each symptom ranges from 1 to 4, and the total score ranges from

20 to 80. A higher score indicates a higher level of anxiety. State anxiety reflects feeling of tension and apprehension associated with increased autonomic nervous system activity at a given time and represents a transitory emotional condition. Trait anxiety exhibits a tendency to perceive stressful situations as threatening and reflects a general emotional condition. The STAI is widely used and has a relatively high level of reliability.[27,28]

Stressors. A mental arithmetic test[25,29] that was done on a computer constituted psychological stress. Each participant was asked to subtract 3-digit numbers from 4-digit numbers (eg, 317 from 1296) as quickly and as accurately as possible in the period between T70 and T75. Upon making a mistake, each participant had to start again from the first subtraction.

Determination of Adrenaline, Noradrenaline and Cortisol Concentrations

The plasma cortisol concentration was determined using a competitive protein-binding assay.[30] Plasma adrenaline and noradrenaline concentrations were determined by high-pressure liquid chromatography with electrochemical detection.[31] The intra- and interassay coefficients of variance were 3.4% and 6.8% for cortisol, 5.0% and 5.2% for adrenaline, and 4.5% and 9.8% for noradrenaline. The limits of assay sensitivity were 3.75 pg for cortisol and 1.00 pg for adrenaline and noradrenaline.

Statistical Analyses

The Mann-Whitney test was used for comparing the 2 mean anxiety levels. Temporal variations of plasma cortisol, adrenaline, and noradrenaline concentrations were evaluated using 2-way analysis of variance (ANOVA) followed by post hoc Bonferroni/Dunn test. The comparison between the basal level and the level at each time point in the same group was evaluated using 1-way ANOVA followed by a post hoc Bonferroni/Dunn test. Spearman rank correlation was used for determining relationships between anxiety levels and the concentrations of cortisol, adrenaline, and noradrenaline. In the analysis of correlations, a change in concentration was defined as the difference between the maximal and minimal concentrations during the experiment. $P < .05$ was considered statistically significant.

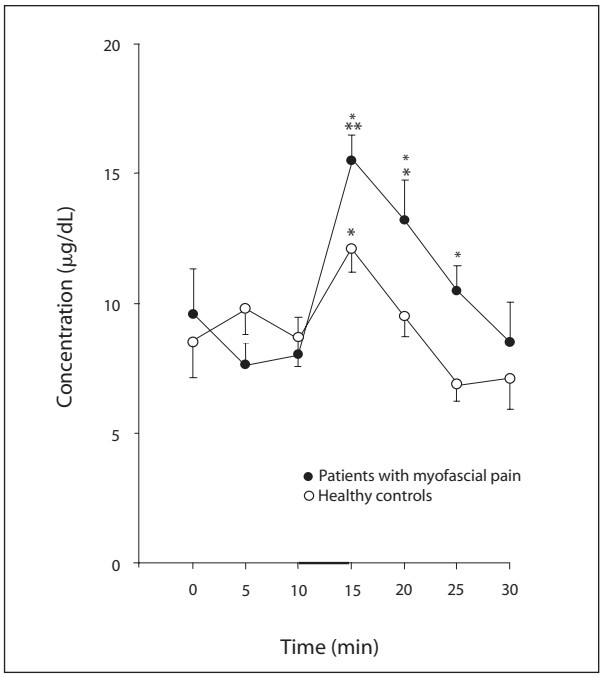

Fig 2 Plasma cortisol concentrations in patients with myofascial pain and healthy controls. The bolded area on the x axis indicates the period of psychological stress. Data are expressed as means ± SE (n = 20). *$P < .05$ for pain patients versus healthy controls. *$P < .05$ for mean plasma cortisol concentration at the time indicated versus at 0 minutes. ** $P < .02$ for mean plasma cortisol concentration at the time indicated versus at 0 minutes.

Results

Anxiety Levels

The mean values ± SE for state anxiety levels in patients with myofascial pain and in healthy controls did not differ significantly (44.10 ± 0.77 and 43.20 ± 0.67, respectively). The mean values for trait anxiety levels in patients with myofascial pain and healthy controls were 50.10 ± 0.61 and 38.40 ± 0.98, respectively. The trait anxiety levels in patients with myofascial pain were significantly higher than those in healthy controls ($P < .001$; Mann-Whitney test).

Plasma Cortisol Concentrations

As shown in Fig 2, in patients with myofascial pain, plasma cortisol concentrations were significantly elevated immediately after the psychological stress period (ie, at 15 minutes or T75; mean ± SE, 15.50 ± 0.97 µg/dL) and 5 minutes later (ie, at 20 minutes; 13.20 ± 1.54 µg/dL) in comparison with

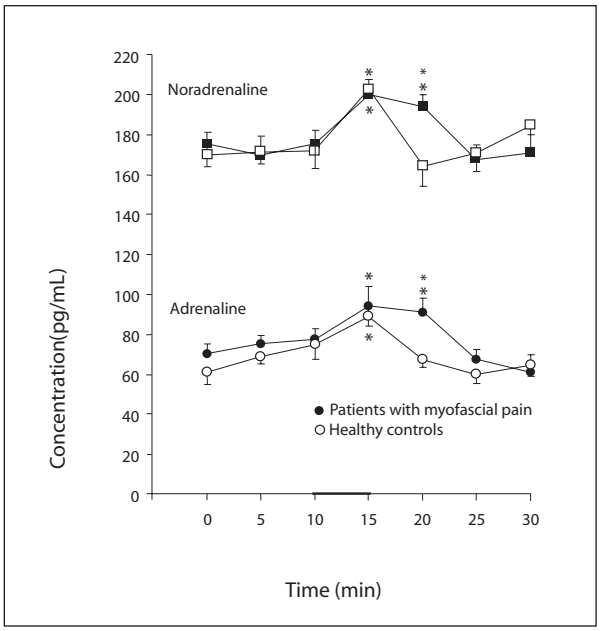

Fig 3 Adrenaline and noradrenaline concentrations in patients with myofascial pain and healthy controls. The bolded area on the x axis indicates the period of psychological stress. Data are expressed as means ± SE (n = 20). *P < .05 for pain patients versus healthy controls. *P < .05 for mean concentration at the time indicated versus at 0 minutes. **P < .01 for mean concentration at the time indicated versus at 0 minutes.

Table 1 Correlations Between Anxiety Levels and Changes in Plasma Cortisol, Adrenaline, and Noradrenaline Concentrations

	Patients with myofascial pain		Healthy individuals	
	State anxiety	Trait anxiety	State anxiety	Trait anxiety
Change in plasma cortisol concentration	0.60 P <.001	0.60 P =.016	0.50 P =.042	0.10 NS
Change in plasma adrenaline concentration	0.50 P =.038	0.62 P <.001	0.64 P <.001	0.32 NS
Change in plasma noradrenaline concentration	0.55 P =.027	0.62 P <.001	0.58 P =.020	0.36 NS

Change in concentrations: Difference between the maximum and minimum concentration during the experiment. NS = not significant.

the concentration at 0 minutes (T60; P = .005 and P = .028, respectively; 1-way ANOVA followed by post hoc Bonferroni/Dunn test). In healthy control subjects, plasma cortisol concentrations were significantly elevated at 15 minutes in comparison to the concentration at 0 minutes (8.50 ± 1.36 μg/dL; P = .015). Plasma cortisol concentrations in the healthy controls at 15, 20, and 25 minutes were 12.10 ± 0.89 μg/dL, 9.50 ± 0.79 μg/dL, and 6.90 ± 0.64 μg/dL, respectively; they were significantly higher in the pain patients than in healthy controls (P = .023, P = .027, and P = .018, respectively; 2-way ANOVA followed by post hoc Bonferroni/Dunn test).

Plasma Adrenaline and Noradrenaline Concentrations

Plasma adrenaline concentrations significantly increased in response to psychological stress at 15 (94.40 ± 9.56 pg/mL) and 20 minutes (91.00 ± 7.16 pg/mL) in the pain patients (P = .037 and P = .028 compared with concentration at 0 minutes) and at 15 minutes (89.20 ± 4.73 pg/mL) in the healthy controls (P = .031), as shown in Fig 3. Plasma adrenaline concentration was significantly higher at 20 minutes in the pain patients than in healthy controls (67.50 ± 3.88 pg/mL; P = .012).

As illustrated in Fig 3, plasma noradrenaline concentrations significantly increased in response to psychological stress at 15 (200.00 ± 7.76 pg/mL) and 20 minutes (194.50 ± 5.89 pg/mL) in the pain patients (P = .022 and P = .036 compared with concentration at 0 minutes) and at 15 minutes (202.80 ± 3.72 pg/mL) in the healthy controls (P = .029). Plasma noradrenaline concentration was significantly higher at 20 minutes in patients with myofascial pain than in healthy controls (164.40 ± 10.05 pg/mL; P = .019).

Correlations Between Anxiety Levels and Changes in Cortisol, Adrenaline, and Noradrenaline Concentrations

As noted in Table 1, state-anxiety levels and changes in cortisol, adrenaline, and noradrenaline concentrations were significantly and positively correlated in the pain patients (r = 0.60, r = 0.50,

and $r = 0.55$, respectively). State-anxiety levels and changes in cortisol, adrenaline, and noradrenaline concentrations were significantly and positively correlated in healthy controls ($r = .50$, $r = .64$, and $r = .58$, respectively). Furthermore, trait-anxiety levels and changes in cortisol, adrenaline, and noradrenaline concentrations were significantly and positively correlated in the pain patients ($r = 0.60$, $r = 0.62$, and $r = 0.62$, respectively) but not in healthy patients.

Discussion

The present study using STAI showed that trait-anxiety levels were significantly higher in patients with myofascial pain than in healthy controls. The finding that psychological factors in patients with myofascial pain differ from those in healthy controls is in agreement with the findings of other studies[32,33] using psychometric tests such as the Minnesota Multiphasic Personality Inventory[34] and the Symptom Check List 90R.[35] On the other hand, there was no significant difference in state-anxiety levels between the 2 groups. The environment in which the experiment is carried out might affect state-anxiety levels, which express feelings of tension, regardless of the presence or absence of myofascial pain.

Although the secretion of cortisol after stress has been shown to be a reliable marker of psychological stress,[36,37] it has been demonstrated that HPA responsiveness to psychosocial stress is influenced by gender,[13] eating,[23,38] age,[18] and menstrual cycle phase.[19] It has also been shown that the secretion of cortisol has a circadian rhythm.[39] Therefore, in order to increase the validity of cortisol concentration in the small sample size (20 patients with myofascial pain and 20 healthy controls) in the present study, the subjects were limited to females of similar age, and the subjects were instructed to refrain from eating or drinking for at least 90 minutes before the start of the experiment. Furthermore, blood sampling was performed during the follicular phase of the menstrual cycle and was started at the same time of the day.

The magnitudes of stress responses expressed as cortisol levels in healthy controls in the present study were smaller than those observed in some experimental studies.[19,38,40] The magnitude and time-course of stress responses of cortisol levels are influenced by stress protocols.[37,38] A mental arithmetic task performed with a computer in a 1-to-1 setting (1 subject and 1 experimenter) lasting 5 minutes was used to produce psychological stress in this study. Other studies have used the Trier Social Stress Test (TSST)[41] consisting mainly of a free speech task and a mental arithmetic task performed in front of an audience for 13 to 15 minutes.[19,38,40] Therefore, the difference between stress responses in the present study and those in other studies might be due to the difference in stress protocols, particularly the difference in stressor strengths. The possibility that a difference in mental arithmetic abilities induced different stress responses cannot be ruled out however, since the subjects had to start again from the first subtraction upon a mistake according to the protocol of this study. Further studies using other psychological stress tests such as the TSST and the Stroop Color-Word Interference Test[42] are needed to exclude the possibility of bias in mental arithmetic ability.

Kirschbaum et al reported that different stress protocols may also induce different reactivities in the HPA and SAM systems.[43] In their study, a stress protocol such as the Stroop Color-Word Interference Test elicited significant SAM activation but not significant HPA activation in a 1-to-1 setting. However, the same task elicited not only SAM activation but also HPA activation when performed in front of an audience. The psychological stress test used in the present study increased activities in both the HPA and SAM systems in patients with myofascial pain and healthy controls, as was shown in other studies.[15,18,20–22,44] However, a difference in timing of induction of responses in the HPA and SAM systems by stress observed in other studies was not seen in this study. The reason for this discrepancy is not clear, but it might be because of the different stress protocols used.

It has been shown that cortisol concentrations were increased more by stress exposure in boys with anxiety disorders than in healthy controls[24] and that patients with high levels of anxiety tended to express more intense neuroendocrine reactions under psychological stress than healthy controls.[25] These studies suggest that anxiety levels are associated with increased secretion of cortisol, adrenaline and noradrenaline after psychological stress. Furthermore, in the present study, the time taken for cortisol, adrenaline, and noradrenaline levels to recover from the stress response differed in the 2 groups. This could indicate that both the HPA and SAM systems are more highly activated in response to psychological stress in patients with myofascial pain than in healthy controls. Higher anxiety levels might be associated with higher sensitivity of both the HPA and SAM systems in patients with myofascial pain.

The present study indicated that there is a difference in state anxiety and trait anxiety in relation to neuroendocrine reactions under the condition of psychological stress. State-anxiety levels and changes in plasma cortisol, adrenaline, and noradrenaline concentrations were significantly correlated in patients with myofascial pain and healthy controls, suggesting that state-anxiety levels might be associated with neuroendocrine reactions regardless of the presence or absence of the pain. Although several studies have tried to uncover possible associations between cortisol stress responses and personality traits such as trait anxiety[45,46] and extraversion,[47] the relationship between HPA axis-related stress responses and personality traits is less consistent. Pruessner et al reported that 1 reason for this inconsistency was the fact that the cortisol stress response reflected a state measure that depended not only on the personality of the subject but also on acquired coping strategies, the probability of success in the given situation, and the relationship between them.[48] The present study also did not indicate the correlations between trait-anxiety levels and stress-induced neuroendocrine responses in healthy controls. However, it is interesting that trait-anxiety levels and changes in plasma cortisol, adrenaline, and noradrenaline concentrations were significantly correlated only in the pain patients. These results suggest that anxiety level, in particular trait-anxiety level, might be associated with higher sensitivity of both HPA and SAM systems in patients with myofascial pain.

In conclusion, the present study indicates that both HPA and SAM systems are more highly activated in response to psychological stress in patients with myofascial pain than in healthy individuals and that a high trait-anxiety level might be closely related to this activation.

Acknowledgments

This study was supported in part by a grant (no. 14571958) from the Ministry of Education, Science, and Culture of Japan.

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