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ABSTRACT

The effect of a long mental stress on the hemodynamics of masticatory muscles has not been investigated to date. We hypothesized some hemodynamic and electromyographic changes in jaw-closure muscles related to sympathetic nervous system activity. While healthy adult female volunteers performed a two-hour mental stress task, electromyographic activity of the temporal and masseteric muscles was recorded, and hemodynamic changes of the masseter muscle were measured non-invasively. Autonomic function was assessed by heart rate spectral analysis. Integrated electromyographic activity of the temporalis muscle, but not the masseter muscle, showed an increase that coincided with the increase in sympathetic nervous activity. In the masseter muscle, despite little change in integrated electromyographic activity, notable changes were found in hemodynamic parameters. These results suggest that hemodynamics of jaw muscles is susceptible to mental stress, implying a potential role in the etiology of jaw muscle dysfunction associated with mental stress.

KEY WORDS: heart rate variability, hemodynamics, near-infrared spectroscopy, masseter, electromyographic activity.

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INTRODUCTION

Vasodilatation and vasoconstriction are fundamental physiological adjustments to metabolic demands of the tissue, and disturbances of intramuscular blood flow can be related to muscle pain (Rasmussen *et al.*, 1977; Lund *et al.*, 1986). Hemodynamic changes, which are affected by the sympathetic nervous system (SNS), are thus relevant to muscle physiology. However, there are only a few reports on non-invasive measurements of intramuscular hemodynamic changes in masticatory muscles during sympathetic-activating tasks (Maekawa *et al.*, 1998, 1999a,b; Tsukiyama *et al.*, 1999). Moreover, the tasks in these studies were cold pressor stimulation tasks, which alter cardiovascular function *via* strong activation of SNS and increase arterial pressure, heart rate, and vascular resistance. Compared with cold pressor stimulation, mental stress has a smaller effect on the SNS (Ng *et al.*, 1994), but it is often encountered in daily life and is considered to be an etiologic factor underlying centrally evoked jaw muscle hyperactivity (Yemm, 1971a,b). Thus, mental stress can cause electromyographic and/or hemodynamic changes in jaw muscles, thereby inducing jaw muscle pain conditions. Further, in our previous study (Hidaka *et al.,* 2004), it has been shown that the masseteric hemodynamic change is closely time-related to mental stress, implying that jaw muscles are susceptible to mental stress. It is therefore worth examining how electromyographic and/or hemodynamic parameters of the jaw muscles behave under mental stress of longer duration, to gain some insight into the etiology of jaw muscle dysfunction.

In addition, activity changes in the parasympathetic nervous system (PNS) and in the SNS were evaluated as autonomic parameters by power spectral analysis of heart rate variability (HRV). This convenient and non-invasive method can discriminate between the changes in the PNS and those in the SNS (Akselrod *et al.*, 1981; Gregoire *et al.*, 1996). The discrimination is valuable because interaction between the PNS and SNS has been increasingly recognized in the tissues of the orofacial region (Izumi, 1999). The purpose of this study was to investigate how electromyographic, hemodynamic, and autonomic parameters change in the masseter muscles of healthy adults performing a long (two-hour) mental task.

MATERIALS & METHODS

Subjects

Twelve healthy female volunteers (mean age ± standard deviation, 25.3 ± 2.1 yrs) participated in the study. They had complete dentition except for third molars, and they had Angle Class I molar relationships without tooth crowding or clinical signs of jaw dysfunction. Women were chosen because many more women than men develop temporomandibular disorders (Pedroni *et al.*, 2003). To reduce variability derived from differences in the phases of the menstrual cycle, all subjects were in the follicular phase of the menstrual cycle on the day of the experiment. All subjects participated after giving informed consent to the

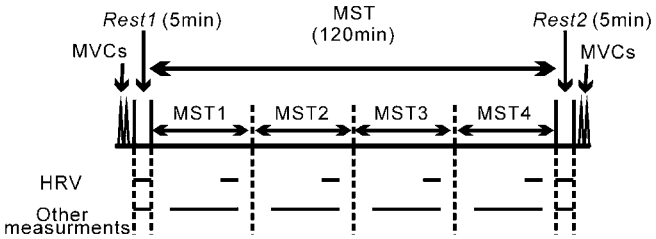


Figure 1. Diagram of the experimental protocol. MST, mental stress task; MVCs, muscle voluntary contractions; HRV, heart rate variability.

protocol that was reviewed and approved by the Ethics Committee of the Faculty of Dentistry.

Mental Stress Task (MST)

The subjects were shown series of 10 Chinese characters. They were requested to pick out, as quickly as they could, a previously given character from each series. The task was performed for 120 min.

Experimental Protocols

The experiments were performed in a noiseless, air-conditioned room. The subjects were instructed to do the following, during which measurements were made: 2 three-second maximum voluntary contractions (MVCs), 5 min of rest (Rest1), 120 min of MST, 5 min of rest (Rest2), and 2 MVCs. At the end of each experimental period, the subjects were asked to report their perceived stress on a scale of: 0, not stressful; 1, somewhat stressful; 2, stressful; 3, very stressful; 4, extremely stressful.

Electrocardiogram (ECG), electromyogram (EMG), and kinetics of masseter muscle oxygenation were continuously recorded. EMG activity was recorded in the superficial masseter and anterior temporalis muscles on both sides. Relative tissue levels of oxygenated hemoglobin (OxyHb), deoxygenated hemoglobin (DeoxyHb), total hemoglobin (TotalHb), and tissue blood oxygen saturation (StO_2) in the left masseter muscle were measured with the use of a laser tissue blood oxygen monitor (BOM-L1TR, Omegawave, Tokyo, Japan).

Data Analysis

The data were digitized by means of an analog-to-digital converter at 2 kHz for the EMG and ECG signals, and at 200 Hz for all the other measurements. The data were analyzed with the use of Spike2 software (Cambridge Electronic Design, Cambridge, UK).

The EMG data were rectified by software and smoothed by a nine-point moving average, and EMG burst was identified as when the EMG activity exceeded a threshold (the mean plus 1 SD calculated in the rest period) for 20 msec. The EMG values were normalized against those during MVC tasks. The integrated EMG burst activity (IEMG) *per* unit time was analyzed.

For the ECG, we determined the R-R interval with a one-millisecond resolution by means of the Spike2 software, which can identify the waveform, and further scanned to detect and correct missing or doubled beats. These data were then interpolated by a cubic-spline function and re-sampled, and the heart rate (HR) power spectrum was analyzed. These procedures were performed by a software package (Time Series Statistical Analyses System: TSAS, University of Tokyo, Tokyo, Japan).

The power spectral components of the R-R interval in the range 0.04-0.15 Hz were defined as the low-frequency (Lo) component, those in the range 0.15-0.50 Hz were defined as the high-frequency (Hi) component, and those in the range 0-0.50 Hz were defined as the total power (TPW) component. The values of Lo/Hi and Hi/TPW were calculated and used as indicators of the SNS and the PNS activities, respectively (Pagani *et al.*, 1986; Butler *et al.*, 1993).

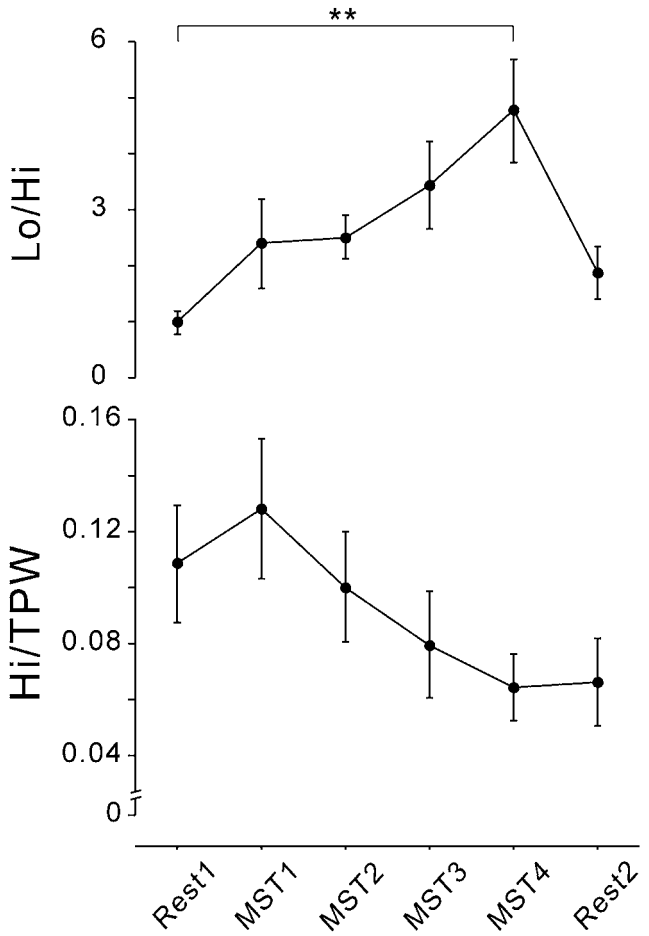


Figure 2. Effects of mental stress on HRV spectral parameters. Lo/Hi, an indicator of the sympathetic nervous system activity; Hi/TPW, an indicator of the parasympathetic nervous system activity; MST, mental stress task. Data are expressed as means ± SEM (standard error of the mean) (n = 12). ** $p < 0.01$ (Scheffé).

The Rest1 period, the MST period divided into 4 30-minute periods (MST 1, 2, 3, and 4), and the Rest2 periods were analyzed (Fig. 1). As for the 4 MST periods, the ECG data were analyzed during the last 5 min, and the other data during the middle 20 min. As for the Rest1 and Rest2 periods, the data were analyzed throughout the 5 min.

Measurements from each analyzed section were expressed relative to the maximum of the measurements obtained from the 6 analyzed periods (100%). The data are expressed as means ± SEM. Differences among the 6 groups were tested by Friedman ANOVA, and the Scheffé *post hoc* test was used to identify differences between groups. $P < 0.05$ was assumed as significant.

RESULTS

Heart rate changed little throughout the 6 analyzed periods, with the mean heart rate ranging only from 72.0 to 74.6 beats

per min (Friedman ANOVA). There were no significant differences in systolic and diastolic blood pressures before and after the mental task (paired *t* test), and all the subjects reported that their perceived stress was at level 4 (extremely stressful) at the end of the experiment.

HRV Spectral Parameter

The HRV spectral parameters varied among the 6 analyzed periods ($p = 0.0005$ for Lo/Hi, and $p = 0.0002$ for Hi/TPW; Friedman ANOVA).

Lo/Hi was higher during MST4 compared with Rest1 ($p = 0.0087$, Scheffé). The increased Lo/Hi tended to decrease after the mental task ($p = 0.0931$ for MST4 *vs.* Rest2, Scheffé), and no more significant increase was found at Rest2 (Fig. 2A). On the other hand, no significant differences in Hi/TPW were found among the 6 analyzed periods (Fig. 2B).

IEMG Activity

IEMG activity of the temporalis muscle varied among the 6 analyzed periods ($p = 0.0049$, Friedman ANOVA), while that of the masseter did not (Fig. 3). The IEMG of the temporalis muscle was higher during MST4 than during Rest1 ($p = 0.0442$, Scheffé).

Hemodynamic Changes of Masseter Muscle

All the hemodynamic parameters varied among the 6 analyzed periods ($p = 0.0150$ for OxyHb, $p = 0.0001$ for DeoxyHb, $p = 0.0021$ for TotalHb, and $p < 0.0001$ for StO_2; Friedman ANOVA) (Fig. 4).

OxyHb was increased during MST1 and MST2 as compared with during Rest1 ($p = 0.0020$ for MST1, $p = 0.0075$ for MST2; Scheffé), whereas DeoxyHb was decreased during MST2, MST3, and MST4 as compared with during Rest1 ($p = 0.0015$ for MST2, $p < 0.0001$ for MST3 and MST4; Scheffé). DeoxyHb showed a reverting tendency after MST, leading to no significant difference between Rest1 and Rest2. TotalHb, the sum of OxyHb and DeoxyHb, tended to be decreased during MST4 as compared with during Rest1 ($p = 0.0963$, Scheffé). StO_2 was increased during MST and Rest2 as compared with during Rest1 ($p = 0.0005$ for MST1, $p < 0.0001$ for MST2, MST3, and MST4, $p = 0.0009$ for Rest2; Scheffé), but showed a reverting tendency after MST ($p = 0.0615$ for MST4 *vs.* Rest2, Scheffé).

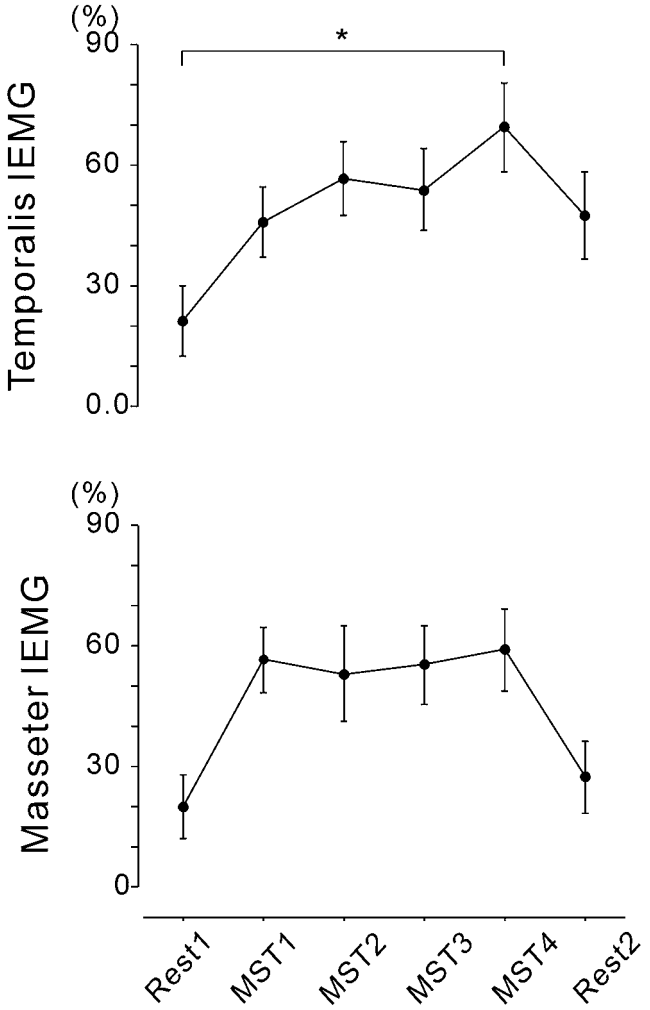


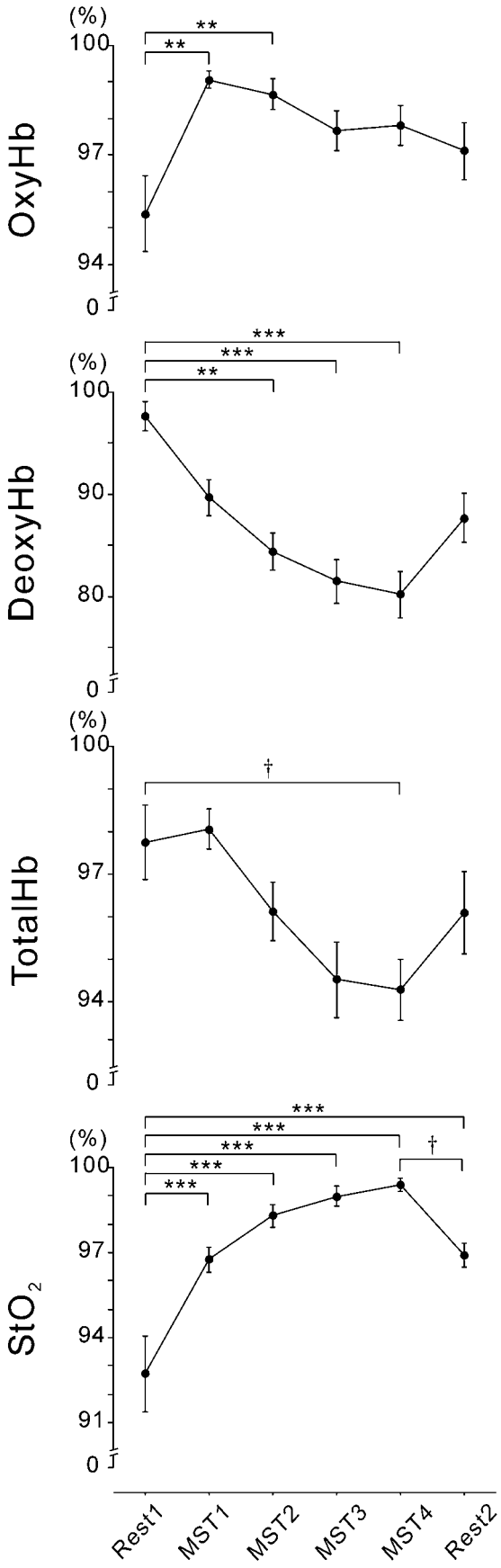
Figure 3. Effects of mental stress on IEMGs of the masseter and temporalis muscles. IEMG, integrated electromyographic activity; MST, mental stress task. Ordinates: values normalized to the maximum of measurements from the 6 analyzed periods (Rest1, Rest2, and MST1-4). Data are expressed as means ± SEM (standard error of the mean) ($n = 12$) * $p < 0.001$ (Scheffé).

DISCUSSION

Sympathetic and Parasympathetic Nervous Activity

Sympathetic and parasympathetic nervous activity is involved in the regulation of blood flow in the tissues of the orofacial region. Sympathetic vasoconstriction, sympathetic vasodilatation, and parasympathetic vasodilatation could be involved in the regulation (Izumi, 1999). In this study, however, parasympathetic nervous activity did not show notable changes in subjects performing a mental task, implying that parasympathetic vasodilatation was not significant in the hemodynamic changes. On the other hand, sympathetic nervous activity increased with time and so may have been involved in the hemodynamic changes. This response from the autonomic nervous system was not as intense as the response in other subjects performing cold pressor tests and other mental tasks, which elicit increases in heart rate and cardiac output (Victor *et al.*, 1987; Freyschuss *et al.*, 1990; Maekawa *et al.*, 1998). In this study, no increase in heart rate was found during the mental test. Thus, the mental task used in this study was a weaker stressor, and hence we could utilize it for a longer period (120 min) than stressors in previous studies. Although the stress in our study is typical of many mental stresses in daily life—in that it is weak and of long duration—to our knowledge, this is the first evaluation of the effect of such mental stress on the autonomic nervous system. From the results of this study, it is reasonable to say that a weaker but longer stressor can increase sympathetic nervous activity without causing an increase in heart rate.

In this study, all the subjects were female. Because it has been suggested that women's physiologic responsiveness to psychologic stress is mediated by their menstrual cycle (Collins *et al.*, 1985), and that autonomic nervous system activation may vary within the cycle (Asso and Braier, 1982), we chose females who were all in the follicular (post-menstrual) phase of the menstrual cycle on the day of the experiment.



IEMG Activity

A notable intermuscle difference in IEMG activity was found. The temporalis muscle showed a time course similar to that of sympathetic nervous activity. On the other hand, the masseter muscle showed little change during the mental task. Fortunately, this is particularly convenient for assessing the hemodynamic changes caused by neural and hormonal mechanisms. This is because muscle contraction causes mechanical compression of the blood vessels and impedes blood flow, competing against the other regulating factors, including local myogenic and metabolic factors (Sejersted *et al.*, 1984; Sjøgaard *et al.*, 1986; Kim *et al.*, 1999).

Hemodynamic Changes in the Masseter Muscle

Hemodynamic changes seem to be related to sympathetic nervous activity. Graphs of DeoxyHb, TotalHb, and StO_2 showed a trough or peak at MST4, where increased sympathetic nervous activity was observed. Blood flow in the tissues of the orofacial region is regulated by autonomic (*i.e.,* sympathetic and parasympathetic) nerves (Izumi, 1999). However, hemodynamic changes observed in this study were most likely caused by changes in the SNS activity, because changes in the PNS activity were small, if any.

The effect of sympathetic nervous activity on the hemodynamics in the masseter muscle, however, is somewhat complex, because changes in TotalHb were not significant. This is not in line with results of previous studies that demonstrated an increase in the Hb concentration of the masseter under cold-pressor stimulation. The discrepancy may be explained by the difference in task characteristics. The cold pressor test elicits pain sensation that can induce nociceptive nerve-mediated vasodilatation (Izumi, 1999), and furthermore, it increases cardiac output (Victor *et al.*, 1987; Maekawa *et al.*, 1998), which increases blood volume through baroreceptor-mediated reflexes (Johnson, 1989). Pain sensation also releases endogenous vasoactive substances (autacoids) such as nitric oxide, eicosanoids, histamine, kinins, adenine nucleotides, and locally produced vasodilator metabolites, all of which can counteract sympathetic vasoconstriction (Cowley and Franchini, 1996). In contrast, the task in this study caused neither pain nor cardiac output increase. This could account for TotalHb tending to show a decrease rather than an increase. Further, the stimulus in this study was longer-lasting. Therefore, the possibility that circulating humoral agents may both impede and potentiate the response to neurogenic vasoconstrictor activity must be considered (Cowley and Franchini, 1996).

OxyHb showed a different pattern of change in peaking at the beginning of the task period. Compared with Rest1, OxyHb increased and DeoxyHb tended to decrease during MST1. The possible hemodynamic changes observed at MST1 might be due to (1) decrease in oxygen consumption in the tissue, (2) alteration in the oxygen dissociation curve of hemoglobin, (3)

Figure 4. Effects of mental stress on hemodynamics of the masseter muscle. OxyHb, oxygenated hemoglobin; DeoxyHb, deoxygenated hemoglobin; TotalHb, total haemoglobin (OxyHb + DeoxyHb); StO_2, tissue blood oxygen saturation (OxyHb/TotalHb x 100); MST, mental stress task. Ordinates: values normalized to the maximum of measurements from the 6 analyzed periods (Rest1, Rest2, and MST1-4). Data are expressed as means ± SEM (standard error of the mean) (n = 12). † $p < 0.1$, ** $p < 0.01$, *** $p < 0.001$ (Scheffé).

changes in blood flow through arteriovenous anastomotic connections, or (4) arterial vasodilatation and venoconstriction due to regional (arteries/arterioles *vs.* veins/venules) variations in vasomotor tone control. Of these, the last one seems most likely, since sympathetic cholinergic vasodilator fibers are confined to arterioles (Cowley and Franchini, 1996). The kinetics of muscle oxygenation in this study seems inconsistent with the findings of our previous study (Hidaka *et al.,* 2004) showing a clear decrease of StO_2 around the onset of mental stress. The discrepancy may be a consequence of the different analytical methods. In the previous study, hemodynamic changes just around the onset of finger sweating were analyzed. On the other hand, in this study, integrals of hemodynamic parameters were obtained from the whole analyzed section, thereby reflecting modulations of the tonic sympathetic activity, the local myogenic and metabolic control, and circulating humoral agent effects.

The muscle hemodynamic change seems sensitive to the activity of sympathetic nerves to the muscle. OxyHb, DeoxyHb, and StO_2 showed a significant change from the first half of the task period, where no significant changes were found in Lo/Hi. In this study, muscle contraction was little if any. Therefore, the hemodynamic change of this study would have been caused exclusively by sympathetic nerve activity, even in the period where Lo/Hi did not show a significant change.

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