# Function of $\beta_2$-Adrenergic Receptors in Chronic Localized Myalgia

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***Aims:*** *To investigate alteration of $\beta_2$-adrenergic receptor ($\beta_2$AR) function in chronic localized myalgia subjects by evaluating levels of the $\beta_2$AR second messenger, cyclic adenosine monophosphate (cAMP), in mononuclear cells after βAR-agonist stimulation.* ***Methods:*** *Eleven chronic localized myalgia subjects and 21 matched healthy controls participated in this study. Peripheral blood (30 cc) was drawn from the subjects' anterocubital vein. Mononuclear cells were isolated from the total blood by using the Ficoll-Hypaque gradient technique. Basal and stimulated intracellular cAMP levels were determined by enzyme immunoassay using a commercially available kit. Aliquots of $5 \times 10^6$ cells were incubated with or without stimulation of the βAR-agonist isoproterenol for 5 minutes. Five different concentrations of isoproterenol ($10^{-3}$M to $10^{-7}$M) were utilized. cAMP levels in both groups were tested statistically by a 2-way repeated-measures ANOVA with 2 predictors, group difference and isoproterenol concentration difference. As with isoproterenol stimulation, the cAMP responses to forskolin, which activates adenylyl cyclase directly and produces cAMP, bypassing the cell surface receptors were also measured.* ***Results:*** *The basal cAMP levels in both groups (myalgia: 0.33 ± 0.02 pmol/$5 \times 10^6$ cells; control: 0.43 ± 0.10 pmol/$5 \times 10^6$ cells) were almost identical, and isoproterenol-produced cAMP levels increased dose-dependently in both groups. No significant differences in the mean cAMP levels were observed between the groups ($P = .909$). Significant increases were observed according to the isoproterenol concentration increase ($P < .0001$). The cAMP responses to forskolin stimulation also showed no significant group difference ($P = .971$).* ***Conclusion:*** *These results suggest that $\beta_2$AR function is not different between localized myalgia subjects and healthy individuals.*

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**Key words:** localized myalgia, $\beta_2$-adrenergic receptor, cAMP, mononuclear cell, isoproterenol

Recent research studies on chronic muscle pain suggest the possibility that long-lasting local pain sensitizes the central nervous system and then turns into persistent chronic pain.[1] However, there has been limited knowledge about the factors that initiate local pain states. Several years ago, localized disturbances in intramuscular blood flow in chronically painful muscle were reported by several researchers and they assumed that this led to hypoxia and accumulation of toxic metabolites, which represented a mechanism for this disorder.[2,3] These results were initially thought to arise from elevated sympathetic activity, which



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might be a contributing factor in the pathogenesis of chronic muscle pain. An alternative explanation was offered by Elam et al, who measured peroneal nerve sympathetic activity in primary fibromyalgia patients and in age-matched controls. They reported no intergroup differences in baseline resting sympathetic activity. Moreover, the patient group did not show increased sympathetic nerve responses (compared to controls) during static hand-grip, jaw muscle contraction, or experimental stress test.[4] These data do not entirely rule out the possibility that some patients might have an exaggerated sympathetic response to normal levels of sympathetic neurotransmitter, only that sympathetic nerves are not firing more frequently in pain subjects.[5]

Therefore, recent studies have suggested that chronic muscle pain is associated with abnormal adrenergic receptor function, not with sympathetic nerve activity itself. Acero et al reported that the intramuscular blood flow increase provoked by cold pressor stimulation, which increases systemic sympathetic neural activity, was significantly diminished in painful muscles compared with those of healthy individuals.[6] This hemodynamic response to cold pressor stimulation in chronic localized muscle pain is very similar to that observed in normal subjects who are intravenously administrated a nonselective β-adrenergic antagonist.[7] In all subtypes of the β-adrenergic receptor (βAR), the $\beta_2$AR is known to be abundantly localized on vascular smooth muscle cells in skeletal muscles,[8] which induces vasodilation when the sympathetic system is activated. The above findings support the notion that $\beta_2$AR activity is diminished in chronic muscle pain patients. Furthermore, research has provided evidence that the $\beta_2$AR is easily desensitized (decrease of its function) or down-regulated (decrease of numbers) by chronic βAR agonist-exposure.[9,10] For these reasons, the authors speculated that a $\beta_2$AR abnormality is associated with chronic muscle pain pathophysiology. In a previous study, we reported that $\beta_2$AR function in mononuclear cells is disturbed in fibromyalgia patients, as demonstrated by evaluating the levels of the $\beta_2$AR second messenger, intracellular cyclic adenosine monophosphate (cAMP), after βAR-agonist stimulation.[11] However, it is still unknown whether this association is also present in patients with localized myalgia. Therefore, the aim of the current study was to compare the mononuclear cell $\beta_2$AR function between individuals who have chronic localized myalgia and matched asymptomatic individuals and to test the null hypothesis that there is no difference in $\beta_2$AR function between these 2 groups.

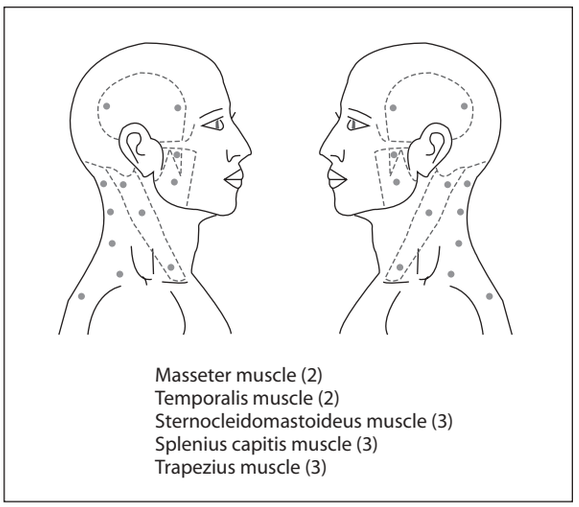


**Fig 1** Orofacial and neck-shoulder muscle palpation sites for selecting participants in this study. Gray circles represent anatomic locations and the numerals in parentheses indicate the number of points that were palpated in each muscle.

## Materials and Methods

### Study Subjects

The study was undertaken on 11 patients with chronic localized myalgia (myalgia group: male/female = 5/6) and 21 asymptomatic subjects (control group: male/female = 10/11). Subjects were selected volunteers from students and staff of the Okayama University Graduate School of Medicine and Dentistry. After a screening questionnaire, which measured resting visual analog scale (VAS) of pain in the orofacial and neck-shoulder region, those patients who were considered suitable for the experiment were verbally questioned about their medical history to ensure that they could safely participate and provide unbiased data for the study. The subjects were then examined clinically to ensure they met the inclusion criteria.

Inclusion criteria for the muscle pain subjects were: *(1)* good physical health, *(2)* pain in the orofacial or neck-shoulder muscle region at least once a week for 6 months, and *(3)* tenderness (more than 30/100 mm response on a VAS) in the orofacial or neck-shoulder muscle region produced by a standardized digital palpation reflecting a steady 19.6 N (2 kgf) compression force for 3 seconds by a well-trained examiner (Fig 1). Inclusion criteria for control subjects were: *(1)* good physical health, *(2)* no history of chronic orofacial or neck-shoulder pain, and *(3)* less than a 10/100 mm VAS response to 19.6



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**Table 1** Mean Physical Characteristics of the Control and Myalgia Groups

| | Control | Myalgia | *P* value |
|---|---|---|---|
| Age (y) | 22.7 ± 1.2 | 22.6 ± 1.2 | .237 |
| Height (cm) | 163.7 ± 8.0 | 164.3 ± 12.3 | .875 |
| Weight (kg) | 58.4 ± 12.4 | 57.5 ± 12.6 | .854 |
| VAS | 0 | 42.3 ± 14.6 | > .001 |
| No. of tender points | 0.4 ± 0.9 | 4.4 ± 3.3 | > .001 |

VAS = visual analog scale of pain (0–100). Data are indicated with ± 1 standard deviation. Statistical analyses were performed with unpaired *t* test, and for VAS data with Mann Whitney *U* test.

N digital compression for 3 seconds in the orofacial or neck-shoulder muscle region. Exclusion criteria for both groups were: *(1)* a current or previous history of fibromyalgia,[12] *(2)* current or previous history of joint or neurologic problems involving the orofacial or neck-shoulder region (eg, degenerative joint diseases, radiculopathy, neuropathic pain conditions), *(3)* a current or previous history of migraine or vascular headache[13] with a frequency greater than 1 per month, *(4)* a current medication or ongoing medical treatment that could influence the results of the study, and *(5)* a current or previous history of illness that is reported to be associated with $\beta_2$AR abnormality, such as asthma, hypertension, cardiovascular disease, and diabetes.[10,14–16] This study protocol was approved by an appropriate ethics committee in Okayama University Dental School (2001-2) and informed consent was obtained from each subject prior to commencement of the study.

### Peripheral Blood Mononuclear Cells (PBMC) Preparation

Peripheral blood (30 cc) was drawn into a heparinized glass vacuum sterile tube from each subject in the evening. Peripheral blood mononuclear cells (PBMC) were separated by a modification of the Ficoll-Hypaque gradient technique of Boyüm.[17] Blood samples were diluted with 30 mL PBS, and each 10 mL sample was layered onto 5 mL of Histopaque (Sigma) and then centrifuged (2,000 rpm, 15 minutes) at room temperature. The white "buffy coat" region, which consists of mononuclear cells, was aspirated, resuspended in AIM-V medium (GIBCO), and washed 3 times at 1,200 rpm for 10 minutes. After centrifugation, the pellet was resuspended in 10 mL of AIM-V medium for cell counting.

### cAMP Accumulation in PBMC

In order to activate $\beta_2$AR and measure the accumulated intracellular cAMP levels in PBMC cells, the βAR-agonist isoproterenol was utilized. In addition, the cAMP response to forskolin, which activates adenylyl cyclase directly and produces cAMP, bypassing the cell surface receptors, was measured.[18,19] Basal levels of intracellular cAMP were measured as well as cAMP accumulation following activation of $\beta_2$AR with isoproterenol and adenylyl cyclase with forskolin, respectively. The PBMC samples were incubated for 10 minutes at 37°C in PBS + 0.05% FBS buffer containing 0.5 mM 3-isobutyl-1-methylxanthine. All compounds were from Sigma Chemical. The PBMC cells were centrifuged (1,200 rpm, 10 minutes) and resuspended in 0.5 mL PBS + 0.05% FBS/$5 \times 10^6$ cells. Then the aliquot of $5 \times 10^6$ cells in each tube was stimulated by 5 different concentrations of isoproterenol ($10^{-3}$ M to $10^{-7}$ M, 5 minutes each concentration) and forskolin ($10^{-3}$ M to $10^{-7}$ M, 10 minutes each concentration). After centrifugation (2,500 rpm, 5 minutes), supernatant was aspirated and the lysis reagent, which is attached to the commercially available kit for cAMP enzyme immunoassay, was added. This facilitated cell lysis and allowed intracellular cAMP levels to be determined in each sample. Each 100 μL of contents of the tubes was collected for the cAMP assay. The intracellular cAMP was expressed as the number of pmol accumulated in $5 \times 10^6$ cells.

### Statistical Analysis

A Student *t* test for unpaired data was performed for comparing the demographic characteristics between the myalgia group and the control group (except for VAS values, for which a Mann-Whitney *U* test was used), while group differences related to intracellular cAMP levels and stimulant concentration were analyzed by 2-way repeated measures ANOVA. A value of $P < .05$ was considered significant.

## Results

### Characteristics and Pain Levels of the Two Groups

Table 1 shows the subjects' physical characteristics. There were no significant differences in mean age, weight, and height between the control and the myalgia groups. However, mean VAS pain levels at rest in the orofacial or neck-shoulder region and the number of tender points under digital muscle palpation were significantly higher in the myalgia group.



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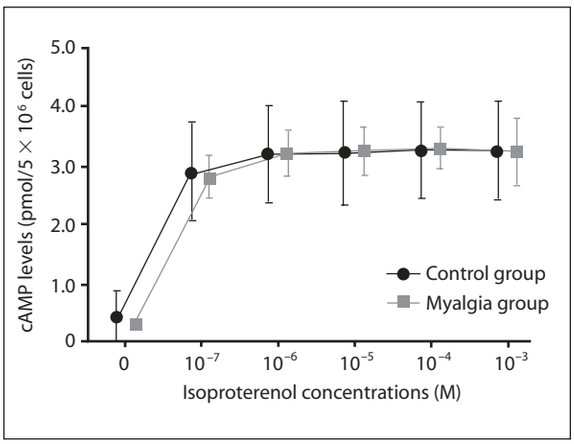


**Fig 2** Mean intracellular cAMP changes induced by isoproterenol stimulation in myalgia and control groups (error bar = standard deviation).

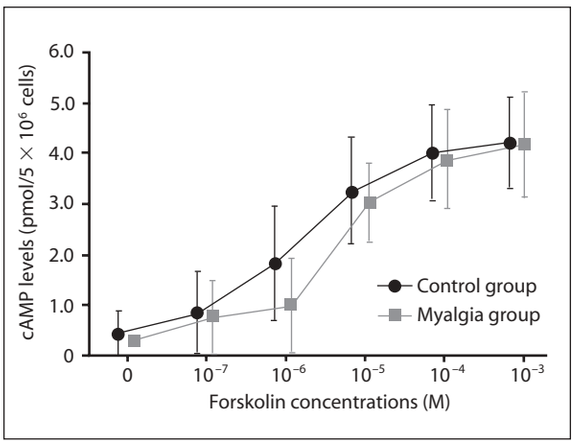


**Fig 3** Mean intracellular cAMP changes induced by forskolin stimulation in myalgia and control groups (error bar = standard deviation).

### Intracellular cAMP Responses

Figure 2 shows the mean intracellular cAMP changes from basal level and after stimulation by different isoproterenol concentrations. The basal cAMP levels in both groups (myalgia: 0.33 ± 0.02 pmol/5 × $10^6$ cells; control: 0.43 ± 0.10 pmol/5 × $10^6$ cells) were almost identical, and isoproterenol-produced cAMP levels increased dose-dependently in both groups. Two-way repeated measures ANOVA revealed that there was no significant mean difference in the cAMP levels between both groups ($P = .909$). A significant cAMP level increase was observed parallel to the isoproterenol concentration increase ($P < .0001$). However, no significant interaction was seen between the group difference and isoproterenol concentration changes ($P = .971$).

As with isoproterenol stimulation, forskolin stimulation did not induce a significant mean difference in the cAMP responses between the 2 groups ($P = .316$) (Fig 3). A significant mean difference was observed according to forskolin concentration increase ($P < .0001$). However, no significant interaction was seen between the group difference and forskolin concentration changes ($P = .390$).

## Discussion

The $\beta_2$AR has been widely studied as a model for the incidence and mechanisms of desensitization and down-regulation.[20] Agonist binding to the $\beta_2$AR causes the receptor to interact with and activate G-protein, which activates adenylyl cyclase. Adenylyl cyclase catalyzes the conversion of adenosine triphosphate (ATP) to cAMP, which in turn activates cAMP-dependent protein kinase, resulting in phosphorylation of particular proteins and specific actions that depend on the cells and tissue type, eg, dilation of vascular smooth muscle. Alterations in the adrenergic response may result from changes at any level of this cascade.

In this study, we evaluated the mononuclear cell $\beta_2$AR responsiveness to the agonist in localized myalgia subjects to test the hypothesis whether localized myalgia subjects have a $\beta_2$AR abnormality (desensitization). Our data have demonstrated that no significant difference in $\beta_2$AR function exists between chronic localized myalgia and asymptomatic subjects. We recognize that the sample size was small and did not have enough power to reject confidently our null hypothesis. Nevertheless, we assessed our data, and given that the mean cAMP levels for the 2 groups were highly and consistently similar at each level of isoproterenol stimulation, we elected to risk a type II analysis error rather than continue collecting data in an effort to raise our power.

Given this limitation, we believe our data suggest that general $\beta_2$AR function is not disturbed in individuals with chronic localized myalgia. This finding also indicates the possibility that the biologic or pathophysiologic background is different between fibromyalgia and localized myalgia, because we have already reported that the function of $\beta_2$AR is diminished in fibromyalgia.[21] Further research is needed to address these issues in more depth and detail.

In addition, this information indicates that the previously disturbed intramuscular hemodynamic response pattern reported in localized myalgia subjects[6] does not induce a general $\beta_2$AR abnormality



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on mononuclear cells. However, since the $\beta_2$AR function measured in this study is not a direct assessment of changes in the painful muscle site, the possibility still exists that an abnormal hemodynamic response occurs to sympathetic provocation within the painful muscle. Additionally, the receptor population, for example, might differ between localized myalgia patients and healthy individuals on vascular smooth muscle cells but not on the PBMC. Further research that evaluates the number and function of local $\beta_2$AR in painful muscle sites is necessary to clarify this point. Moreover, studies that evaluate the biologic effect of vasoactive substances are also needed to elucidate the mechanisms of abnormal intramuscular hemodynamic responses in localized myalgia.

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