Function of ß-Adrenergic Receptors on Mononuclear Cells in Female Patients with Fibromyalgia

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ABSTRACT. ***Objective***. To investigate the ß-adrenergic receptors (ßAR) in patients with chronic fibromyalgia syndrome (FM). These receptors are present on circulating mononuclear cells, and activation of G-protein coupled receptors like ßAR leads to an increase in the intracellular level of cyclic aminosine monophosphate (cAMP). Therefore, cAMP levels can be used to indirectly assess the functional status of the receptor.

Methods. Eight female patients with FM and 9 matched healthy female controls participated in this study. Blood samples were drawn from subjects' anterocubital vein in the morning. Mononuclear cells were isolated from the whole blood according to Boyüm's method. Basal and stimulated intracellular cAMP levels were determined by enzyme immunoassay. Aliquots of 10^6 cells were incubated with or without stimulation of ß-agonist isoproterenol for 5 min. Two different concentrations of isoproterenol (10^{-3} M and 10^{-5} M) were utilized.

Results. The basal cAMP levels in patients with FM (3.02 ± 0.44 pmol/10^6 cells) were slightly more elevated (but not statistically different; $p = 0.124$, Mann-Whitney U test) than that of the control group (2.26 ± 0.39 pmol/10^6 cells). Proterenol 10^{-3} M stimulation significantly increased intracellular cAMP from the basal levels in both groups (FM group, $p = 0.008$; control group, $p = 0.011$). However, isoproterenol 10^{-5} M did not increase mean intracellular cAMP levels in the FM group ($p = 0.74$), while a significant increase was observed in the control group ($p = 0.012$).

Conclusion. These preliminary results suggest that diminished ßAR function is associated with the chronic FM state. (J Rheumatol 2003;30:364–8)

Key Indexing Terms:
FIBROMYALGIA ß-ADRENERGIC RECEPTOR cAMP

Although significant effort has been devoted to determining the pathophysiology of fibromyalgia syndrome (FM), the exact mechanisms have still not been established. Recent studies suggest that FM has a possible connection with abnormal sympathetic system function. Bengtsson and Bengtsson[1] first speculated that there would be excess sympathetic activity in FM and looked at the sympathetic influence on these patients. They evaluated the effect of sympathetic blockade on resting muscle pain and number of tender points and reported that there was a marked reduction in the total number of tender points and pain levels. Bäckman, *et al* used needle electromyography to determine the muscle relaxation rate after a brief electrical stimulation in patients with primary FM[2]. They reported that a slower relaxation rate existed in patients compared with normal controls. They also reported that this slower rate increased toward a normal level following sympathetic blockade. These 2 findings support the sympathetic hyperactivity theory in patients with FM.

Contradictory data were provided by Elam, *et al* and by Yunus, *et al*. Specifically, Elam, *et al* evaluated sympathetic nerve activity in the peroneal nerve using a direct neuronal recording method at rest in patients with FM and age matched controls[3]. They did not observe clear group differences in baseline sympathetic activity. Yunus, *et al* measured plasma and urinary catecholamine levels as a marker of sympathetic nerve activity in each group but failed to observe significant differences in any of the catecholamine measures[4]. These findings suggest that the relationship between sympathetic nerve activity itself and pathophysiology of FM is unclear.

Regarding other variables that reflect sympathetic responsiveness, there have been several reports that patients with FM have abnormal cardiovascular hemodynamics. Bou-Holaigah, *et al* tested the abnormal responses to upright tilt table testing in 20 patients with FM and 20 healthy controls[5]. They found that 60% of the FM patients but no controls had an abnormal drop in blood pressure with upright tilting. They also reported that the mean heart rate increased during upright challenge in controls but it did not change in patients with FM. Martínez-

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Lavín, *et al* assessed the sympathetic-parasympathetic balance in individuals with FM by power spectral analysis of heart rate variability, which is defined as the amount of heart rate fluctuation around the mean heart rate[6,7]. They found significant differences between the patients with FM and healthy controls in the low frequency band of this analysis[6]. In addition, they also discovered that FM patients had diminished accumulated 24 hour heart rate variability[7]. Raj, *et al* also reported similar findings that show less heart rate variability in FM patients[8].

Following the findings reported by Martínez-Lavín, *et al*[6,7], Kelemen, *et al* assessed heart period variability, which shows the balance between levels of activity of the cardiac sympathetic and parasympathetic nerves[9]. They reported that patients with FM had an impaired baroreflex, which might suggest that the abnormal sympathovagal balance was capable of blunting expression of a further sympathetic activation. In order to test the sex difference of autonomic function in FM patients, Cohen, *et al* measured cardiovascular variables using power spectrum analysis of heart rate variability[10]. They demonstrated that male patients with FM at rest are characterized by sympathetic hyperactivity and concomitantly reduced parasympathetic activity. An abnormal sympathovagal response was observed during postural changes. However, this study showed that female patients with FM exhibit more augmented sympathetic activity and reduced vagal tone than male patients. Cohen, *et al* also obtained short term (20 min complete rest at spine position) electrocardiogram recordings and observed significantly higher heart rate and significantly lower heart rate variability in FM patients than healthy controls[11].

All these studies further support the notion that an altered sympathetic responsiveness is present in FM; the cardiovascular data suggest a desensitization of the adrenergic receptors may be present[12]. If so, even elevated levels of adrenergic neurotransmitters (i.e., norepinephrine or epinephrine) will not produce the expected sympathetic response.

We examined the ß-adrenergic receptor (ßAR) activity in patients with FM. Our selection of this receptor is based on the above data, and that all subtypes of ßAR are well known to easily undergo desensitization or to be downregulated by chronic ßAR agonist stimulation. Additionally, it is also known that $ß_2$AR are abundant in the smooth muscle cells of skeletal muscle vessels, which control the blood vessel diameter and influence cardiac function. Since the characteristics of $ß_2$AR on circulating mononuclear cells are highly correlated to the $ß_2$AR on solid tissues such as skeletal muscle[13], the $ß_2$AR on mononuclear cells will be a good marker to evaluate the systemic $ß_2$AR characteristics. Specifically we compare $ß_2$AR characteristics in mononuclear cells derived from peripheral blood in FM patients and those in healthy controls.

MATERIALS AND METHODS

Study subjects. Subjects were recruited by local newspaper advertisement and by sending a study notice to local fibromyalgia patient self-help groups in the Los Angeles area. After a screening questionnaire, those people deemed suitable were questioned verbally about their medical history to ensure that they could safely participate in the experiment and provide unbiased data. Informed consent was obtained before the start of the experiment, and a consent form approved by the Human Subjects Protection Committee of UCLA was signed by each participant.

The criteria for inclusion in the non-FM healthy control group were as follows: (1) Self-rated as being in good physical health; and (2) female between the ages of 21 and 55 years. FM group inclusion criteria were as follows: (1) self-rated as being in fair to good physical health (except for their FM); (2) female between the ages of 21 and 55 years; (3) duration of FM of over 6 months; and (4) a moderate tenderness response at at least 11 of 18 muscle palpation sites according to the American College of Rheumatology (ACR) criteria[14]. Subjects' exclusion criteria for this study were: (1) current or previous history of any substantial medical problems; (2) current ongoing medical treatment for an illness other than FM or its associated symptoms; and (3) taking any prescription or nonprescription medications.

Peripheral blood mononuclear cell preparation. Peripheral blood (20 ml) was obtained from each subject in the morning. Blood was drawn into a glass vacuum sterile tube containing EDTA. Peripheral blood mononuclear cells (PBMC) were separated using a modification of the Ficoll-Hypaque gradient technique of Boyüm[15]. Blood samples were centrifuged (1500 rpm, 15 min) at room temperature. White mononuclear cell layer "buffy coat" region was diluted by phosphate buffered saline (PBS) and carefully layered onto the 3 ml of Histopaque (Sigma) and then centrifuged (2000 rpm, 10 min). Mononuclear cell bands were aspirated, resuspended in AIM-V medium (Gibco), and washed 3 times at 1200 rpm for 10 min. After centrifugation, the pellet was resuspended in 1 ml of AIM-V medium for cell counting.

cAMP accumulation in PBMC. Basal levels of intracellular cAMP were measured as well as cAMP accumulation following activation of $ß_2$AR and adenylate cyclase with isoproterenol and forskolin, respectively. PBMC samples were incubated for 10 min at 37ºC in PBS + 0.05% fetal bovine serum (FBS) buffer containing 0.5 mM 3-isobutyl-1-methylxanthine. All compounds were from Sigma Chemical Co. (St. Louis, MO, USA). The PBMC cells were centrifuged (1200 rpm, 10 min) and resuspended in 0.5 ml PBS + 0.05% FBS/10^6 cells. Then aliquot 10^6 cells to each tube was stimulated by isoproterenol (10^{-3} M and 10^{-5} M, 5 min) and forskolin (10^{-5} M and 10^{-7} M, 10 min), respectively. After centrifugation (2500 rpm, 5 min), supernatant was aspirated and lysis reagent, which is attached to the commercial kit for cAMP enzyme immunoassay (Amersham Pharmacia Biotech, Arlington Heights, IL, USA), was added. This facilitates cell lysis and determination of intracellular cAMP level. Each 100 µl of contents of tubes was collected for the cAMP assay. The intracellular cAMP was expressed as the number of pmol accumulated in 10^6 cells.

Statistical analysis. A Student t test for unpaired data was performed for comparing demographic characteristics between the FM group and control group. Since the sample size in the study was too small to utilize parametric testing, differences of basal intracellular cAMP levels between the groups were analyzed by Mann-Whitney U test. The intracellular cAMP changes from the basal level in each group were analyzed by Wilcoxon signed rank test. A value of $p < 0.05$ was considered to be significant.

RESULTS

Characteristics of the 2 groups. Table 1 shows the subjects' physical characteristics and the number of tender points by digital palpation according to ACR criteria[14]. While the 2 groups had different mean ages, with the FM subjects being 7.5 years older, statistical analysis showed this difference did not achieve significance. Moreover, for the other physical characteristics of weight and height no difference existed between the control and the FM groups. For the purposes of this preliminary report, we elected to proceed with the analysis rather than collect additional data towards the goal of

Table 1. Demographic characteristics of controls and FM patients. Data are means.

	Controls	FM Group	p
Age, yrs	35.8	43.3	0.075
Height, ft	5.44	5.51	0.35
Weight, lbs	136	138.6	0.846
No. of tender points	3	15.3	< 0.0001

p value was calculated with unpaired t test. Tender points were evaluated according to American College of Rheumatology criteria[14].

reducing the age discrepancy. As would be expected, significant differences were observed in number of tender points between the groups. Mean duration of FM in the patient group was 10.6 years.

Intracellular cAMP responses. As shown in Figure 1, the mean intracellular cAMP levels of the basal condition (before stimulation) in the FM group were slightly more elevated than controls, but not statistically different (p = 0.124; Mann-Whitney U test). Isoproterenol 10^{-3} M stimulation significantly increased intracellular cAMP from the basal levels in both groups (FM group, p = 0.008; control group, p = 0.011) (Figure 1). However, isoproterenol 10^{-5} M did not increase mean intracellular cAMP levels in the FM group (p = 0.74), while significant increase was observed in the control group (p = 0.012). Regarding forskolin stimulation, the 2 different (low and high) concentrations significantly increased intracellular cAMP levels in both groups (Figure 2).

DISCUSSION

The $ß_2$AR has been widely studied as a model for the incidence and mechanism of desensitization and downregulation of receptors[16]. Agonist binding to the $ß_2$AR causes the receptor to interact with and activate the G-protein, which activates adenylyl cyclase. Adenylyl cyclase catalyzes the conversion of adenosine triphosphate (ATP) to cAMP. cAMP activates the cAMP dependent protein kinase, resulting in phosphorylation of particular proteins and specific actions that depend on the cells and tissue type, e.g., dilation of vascular smooth muscle. Alteration in adrenergic response may result from changes at any level of this cascade.

We evaluated mononuclear cell $ß_2$AR responsiveness to the agonist in female FM patients to test the hypothesis that FM patients have a $ß_2$AR abnormality (desensitization). In resting conditions (no stimulation of the receptor), intracellular cAMP levels showed no significant difference between FM and control groups. However, the responses to ßAR-agonist stimulation were different between the 2 groups. At a high concentration (10^{-3} M) level of isoproterenol stimulation, intracellular cAMP levels significantly increased from the basal levels in both groups. On the other hand, the low concentration (10^{-5} M) level of isoproterenol produced a significant increase in cAMP in the control group, but no significant mean increase was observed in the FM group. Interestingly, the cAMP response to the forskolin, which bypasses the cell surface receptors and activates adenylyl cyclase, was not blunted in either group. These findings indicate the possibility that $ß_2$AR function itself is disturbed, through homologous desensitization, in patients with FM. Several researchers have reported that blunted cAMP responses are associated with reduced $ß_2$AR numbers[17-19], however, the presence of fewer $ß_2$AR may also be associated with homologus desensitization. Additional studies to evaluate the number of $ß_2$AR in patients with FM are needed. Another mechanism that would explain the blunted cAMP response in FM is a reduction in the levels of G-protein[20]. If the number or the function of G-protein is reduced in cells, all G-protein coupled receptors are desensitized on the surface of the cells, which means heterologous

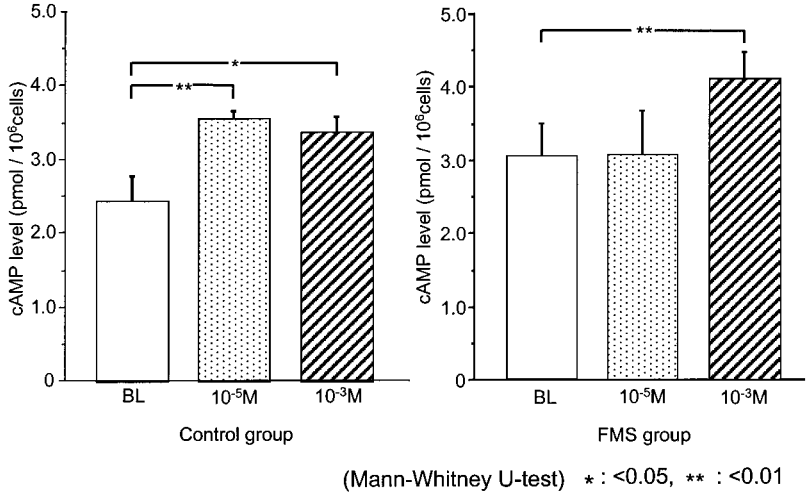

Figure 1. Mean cAMP levels after isoproterenol stimulation in control and FM groups. BL: baseline; 10^{-5} M: after 10^{-5} M isoproterenol stimulation; 10^{-3} M: after 10^{-3} M isoproterenol stimulation.

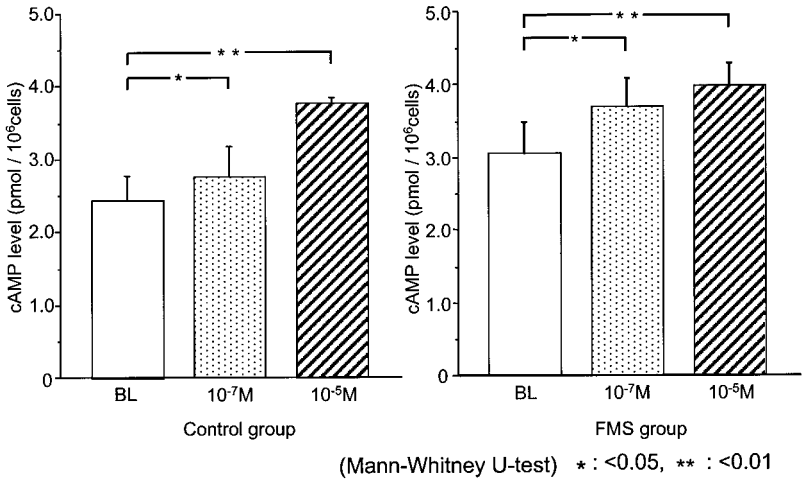

Figure 2. Mean cAMP levels after forskolin stimulation in control and FM groups. BL: baseline; 10^{-7} M: after 10^{-7} M forskolin stimulation; 10^{-5} M: after 10^{-5} M forskolin stimulation.

desensitization. The findings reported here also suggest a need for further detailed studies of G-proteins in patients with FM.

Similar studies of cAMP responses have been conducted in mononuclear or lymphocyte cells from the depressed patients. They found blunting of cAMP responses to isoproterenol[21-24], except for one group[25]. These reports indicate the possibility that the findings reported here are due to the depressed state in our subjects, since it is well known that patients with FM often have depression[26,27]. Unfortunately, we did not measure the psychological states of the subjects in this study. Future studies comparing cAMP responses between FM patients with or without depression will be needed to clarify this point. However, since recent studies provided the evidence that administration of antidepressants provokes consistent postsynaptic ßAR receptor downregulation in the central nervous system[28], the above results of blunted cAMP responses in depressive patients might be caused by antidepressant effect, not a depressed state itself. Since we selected subjects who were taking no medication in this study, it is considered that our results are more likely associated with the FM state than with another comorbid condition.

We observed evidence of abnormal $ß_2$AR responses in patients with FM. These findings are important because it is possible to consider that previously reported abnormal sympathetic responses in FM patients, e.g., blunted cardiovascular responses upon sympathetic stress, are explainable with these findings. This becomes an explanatory model of the central and peripheral abnormal cardiovascular function in FM with the study of Elam, *et al*[3] and Yunus, *et al*[4], which showed no significant sympathetic nerve activities and produced similar catecholamine levels in FM patients and normal controls. That is to say, while sympathetic nerve activities (catecholamine production) show the same level in both groups, the relative functional effect on cardiovascular systems could be suppressed because of a diminished $ß_2$AR function in patients with FM.

Our results provide reasonable evidence for an association between the FM disease state and abnormal $ß_2$AR function. It is not clear why and how this association arises and which role it plays in FM pathophysiology. Further studies with larger samples are required to confirm the role of the $ß_2$AR abnormality in patients with FM.

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