Increased Plasma and Joint Tissue Adrenomedullin Concentrations in Patients with Rheumatoid Arthritis Compared to Those with Osteoarthritis

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ABSTRACT. ***Objective.*** To elucidate the pathophysiological role of adrenomedullin (AM) in rheumatoid arthritis (RA), plasma AM concentration was measured in patients with RA and in healthy contols. The concentration of AM in joint fluid, synovial tissue, and articular cartilage of patients with RA and osteoarthritis (OA) were measured and compared.

Methods. Twenty-six patients with RA (aged 62 ± 4 yrs, all female), 10 healthy controls (aged 57 ± 5 yrs, all female), and 10 patients with OA (aged 68 ± 8 yrs, all female) were studied. We measured plasma levels of total and mature AM by immunoradiometric assay and levels of AM in joint tissue by radioimmunoassay.

Results. Plasma levels of AM in patients with RA (18.35 ± 6.9 fmol/ml) were found to exceed those in healthy controls (11.64 ± 2.8 fmol/ml). Moreover, plasma AM showed a significant positive correlation with plasma C-reactive protein (CRP). The correlation coefficient of total AM was 0.685, and that of mature AM was 0.624. Similarly, AM levels in synovium and joint fluid in patients with RA were significantly higher than in OA. In contrast, AM levels in articular cartilage were found to be low, with no significant difference in levels between patients with RA and OA.

Conclusion. The relation between plasma AM levels and plasma CRP in patients with RA suggests that plasma AM levels increase with the activity of RA. Moreover, AM levels in synovium and joint fluid of patients with RA were significantly higher than those of patients with OA. Thus, AM probably plays a part in the regulation of the inflammatory process of RA. (J Rheumatol 2003;30:2553–6)

Key Indexing Terms:
ADRENOMEDULLIN JOINT FLUID OSTEOARTHRITIS
RHEUMATOID ARTHRITIS SYNOVIUM

Adrenomedullin (AM) is a 52 amino acid peptide, which was identified in human pheochromocytoma using elevated platelet cAMP activity as an indicator[1]. AM exerts a potent hypotensive effect in several species. Besides its potent vasodilatory action, AM might play an important role in the regulation of inflammatory processes[2-4]. Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory disorder affecting multiple joints. While plasma AM concentrations have been reported to be elevated in rheumatic disorders[5,6], the role of AM in RA has not been clarified.

To determine the role of AM in RA, we investigated the correlation between plasma AM concentrations and plasma C-reactive protein (CRP) concentrations in patients with RA using immunoradiometric assay (IRMA). In addition, AM levels in joint tissues such as joint fluid, synovium, and articular cartilage were measured by radioimmunoassay (RIA).

Two molecular forms of AM circulate in human plasma: an active, mature form (mature AM) and an intermediate, inactive, glycine-extended form (AM-Gly). Recently, a new one-step direct immunoradiometric assay system was developed for mature AM and total AM (mature AM + AM-Gly)[7]. The IRMA system was developed to measure the AM concentration in plasma; IRMA makes it possible to specifically measure mature AM, using a small amount of plasma sample, by a one-step overnight assay without prior extraction[7].

We first describe the plasma AM levels in RA patients compared to those of healthy controls, and then the correlation between plasma AM levels and CRP levels in patients with RA. Additionally, we compare AM levels in joint tissues between patients with RA and those with OA by RIA as described[8].

MATERIALS AND METHODS

Patient characteristics and preparation of samples. For measurement of plasma AM, the study population consisted of 26 patients with RA aged 58–73 years (mean ± SD 62 ± 4 yrs), 10 with OA aged 59–76 years (68 ±

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8), and 10 healthy volunteer controls aged 50–66 years (57 ± 5) (Table 1). all patients and controls gave their informed consent. Joint fluid, synovial tissue, and cartilage were acquired from surgical subjects during total knee arthroplasty in patients with RA (n = 6) and OA (n = 6). Patients in all groups were female. All patients with RA were classified as stage 4, class 2, according to the criteria of the American Rheumatism Association (ARA), while all patients with OA were classified as stage 4 to 5, according to the Kellgren-Lawrence radiographic staging system[9]. Since plasma AM concentration has been reported to be elevated in patients with hypertension, renal failure, systemic infections, myocardial infarction, and heart failure[4,10-12], patients and controls with these conditions were excluded.

Measurement of total and mature AM in plasma. Whole-blood samples (total 7 ml) were taken from a peripheral vein using a 25-gauge needle with the patient in a supine position early in the morning after overnight fasting. Blood samples were transferred into tubes containing 1 mg/ml EDTA-2Na and 500 kallikrein inhibitory units/ml of aprotinin for measurement of AM. The plasma was kept at –30°C until assayed.

Levels of mature AM and total AM were measured by IRMA using specific kits (AM mature RIA, AM RIA Shionogi) developed by Shionogi Pharmaceutical Co. Ltd., Osaka, Japan[7,13]. The limit of detection of human mature-AM or total-AM is 0.5 pmol/l for both kits.

Extraction of AM in joint tissues. For measuring AM levels of acquired joint fluids, samples were acidified with acetic acid to a final concentration of 1.0 M and centrifuged at 3000 rpm for 5 min, while synovium and cartilage specimens were acidified with acetic acid to a final concentration of 1.0 M and boiled for 10 min to inactivate proteases. The samples were then homogenized, and centrifuged for 90 min at 12,000 rpm.

The supernatant of samples was applied to a Sep-Pak C18 cartridge (Millipore-Waters, Milford, MA, USA). After the cartridge was washed with 10% CH_3CN in 0.1% trifluoroacetic acid, the absorbed materials were eluted with 50% CH_3CN in 0.1% trifluoroacetic acid. The eluted samples were dried by speed vacuum, freeze-dried, and stored at –30°C until assayed.

Radioimmunoassay for total AM. The RIA for total AM was performed as described[8]. The incubation buffer for RIA was 0.05 M sodium phosphate buffer (pH 7.4), containing 0.5% BSA, 0.5% Triton X-100, 0.08 M NaCl, 0.025 M EDTA 2Na, 0.05% NaN_3, and 500 KIU/ml trasylol. A disposable plastic tube (10 × 75 mm) was used for assay. All assay procedures were performed at 4°C. Both standard AM and unknown samples (100 µl) were incubated with anti-AM antiserum diluent (200 µl) for 12 h before the tracer solution (^{125}I-AM, 18,000–20,000 counts/min in 100 µl) was added. After incubation for 16 h, anti-rabbit IgG goat serum diluent (100 µl) was added. After resting for 24 h, the tubes were centrifuged at 3000 rpm for 30 min at 4°C and the radioactivity of the precipitate was measured by an Aloka ARC-600 gamma counter.

Statistical analysis. All data were expressed as means ± SD. Differences between 2 groups were analyzed using the unpaired t test. Multiple comparisons were assessed with one-way analysis of variance followed by Scheffe's test. Linear regression analysis was used to assess the correlation between variables. A p value < 0.05 was considered statistically significant.

RESULTS

Patients with RA demonstrated high plasma concentration of total AM (18.35 ± 6.9 fmol/ml) compared to healthy controls (11.64 ± 2.8 fmol/ml) and OA patients (12.88 ± 1.9 fmol/ml) (Table 2). Total and mature AM increased in parallel in patients with RA and in healthy controls, with a correlation coefficient of 0.78 (Figure 1). Further, plasma AM (total and mature) and plasma CRP levels were found to be well correlated (Figures 2 and 3). The correlation coefficient between CRP and total AM was 0.685, and that of mature AM was 0.624.

AM (total AM) concentration in the joint fluid of RA patients (10.8 ± 4.3 fmol/ml) was significantly higher than that of OA patients (7.2 ± 1.8 fmol/ml) (Figure 4). The AM concentration in synovium of RA patients was significantly higher than that of OA patients, at 156 ± 35 and 51 ± 9 fmol/g, respectively ($p < 0.01$) (Figure 5).

In contrast to joint fluid and synovium, AM concentration in articular cartilage of patients with RA was considerably lower (37 ± 0.8 fmol/g), and was not statistically significant compared with the rate of 28 ± 0.4 fmol/g in OA (Figure 5).

Table 1. Characteristics of patients with RA and OA and control subjects.

	Age, yrs	Stage
RA patients, n = 26	62 ± 4	Stage 4 class 2
OA patients, n = 10	68 ± 8	Stage 4 to 5
Controls, n = 10	57 ± 5	

All values are expressed as means ± SD.

DISCUSSION

It has been reported that some collagenous disorders show increased levels of plasma AM[5]. We measured and compared plasma AM concentrations in patients with RA

Table 2. Plasma adrenomedullin (AM) levels.

	Total AM, fmol/ml	Mature AM, fmol/ml
RA patients	18.35 ± 6.9*†	1.80 ± 1.4*†
OA patients	12.88 ± 1.9*	1.42 ± 0.8*
Controls	11.64 ± 2.8†	1.34 ± 0.9†

All values are expressed as means ± SD. * $p < 0.01$, † $p < 0.01$.

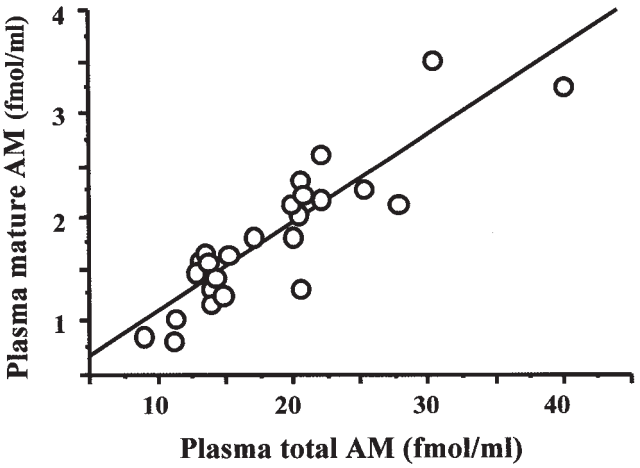

Figure 1. Correlation of mature AM and total AM in plasma in patients with RA. A significant positive correlation was observed between mature AM and total AM (correlation coefficient = 0.78, $p < 0.01$).

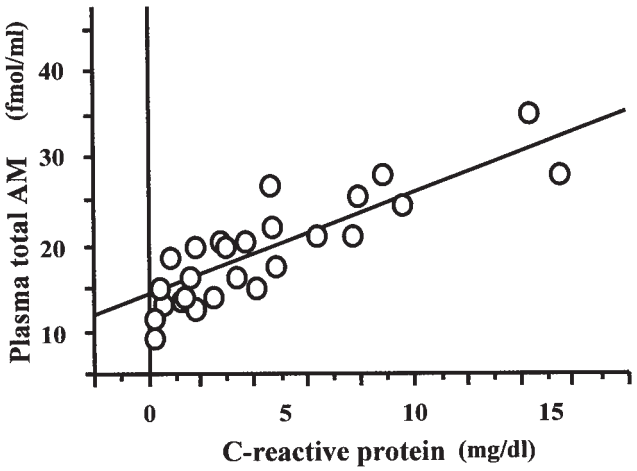

Figure 2. Correlation between total AM and CRP. A significant positive correlation was observed between AM and CRP (correlation coefficient = 0.685, $p < 0.01$).

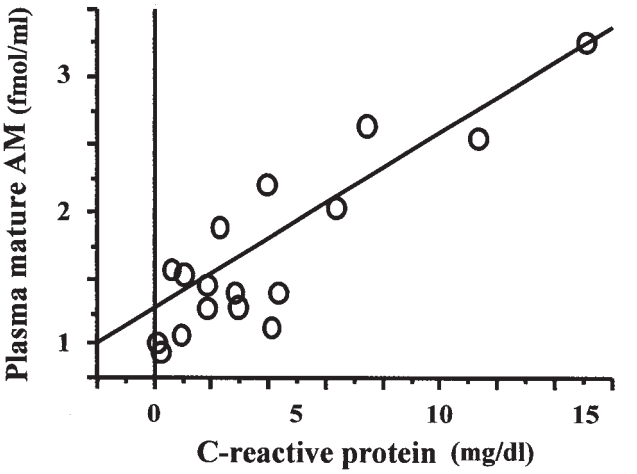

Figure 3. Correlation between mature AM and CRP. A significant positive correlation was observed between mature AM and CRP (correlation coefficient = 0.624, $p < 0.01$).

and healthy controls, finding that patients with RA exhibited a 1.7-fold increase in plasma total and mature AM levels (Table 2). In this study, mature and total AM showed corresponding increases in the plasma (Figure 1).

With respect to the plasma mature/total AM ratio, there were no significant differences among patients with RA or OA, and control subjects (Table 2). Kitamura, *et al* also reported that most of the total AM represents immunoreactivity of AM-Gly and that the concentration of immunoreactive mature AM in plasma is much lower than that of AM-Gly[14].

Plasma AM levels in patients with RA were also found to have a significant correlation to CRP levels (Figures 2 and 3). In RA, CRP correlates with disease activity and response to therapy. Our results suggest that AM levels are increased in patients with RA and that they might be correlated with disease activity.

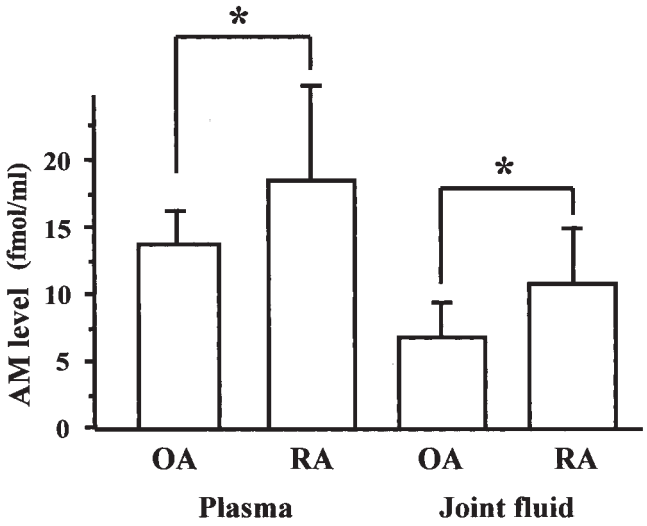

Figure 4. Concentration of AM (total AM) between RA and OA in plasma and joint fluid. Patients with RA had higher plasma and joint fluid concentrations of AM compared to OA patients. *$p < 0.01$.

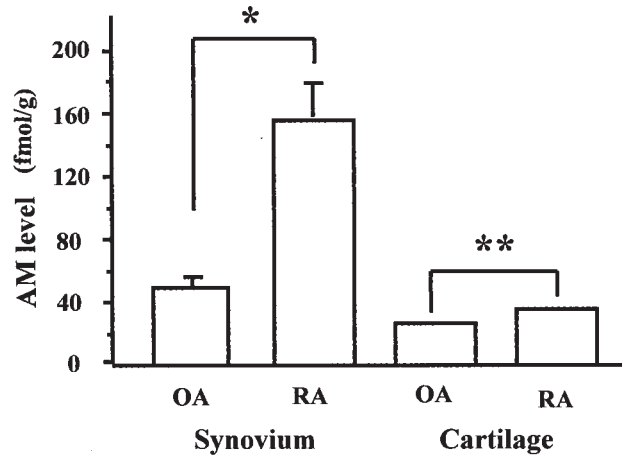

Figure 5. Concentration of AM for synovium and articular cartilage in patients with OA and RA. RA patients showed higher concentrations of AM in synovium compared to OA patients. *$p < 0.01$; **not significant.

The IRMA has been developed to measure plasma concentration of total and mature AM, and we applied conventional RIA to determine AM concentration in tissues. RA is characterized by the presence of an inflammatory synovitis accompanied by destruction of joint cartilage and bone. The concentration of AM in synovium of RA patients was 3.2-fold higher than that of OA patients (Figure 5). As well, the concentration of AM in plasma of RA patients was 1.4-fold higher than that of OA patients (Table 2).

To determine the relation between plasma AM and arthritis in patients with RA, we compared AM levels in joint fluid of RA and OA patients. Joint fluid is similar in composition to plasma, which explains the similarly significant increases in AM concentration in both plasma and joint

fluid in patients with RA compared to those with OA ($p < 0.01$) (Figure 4). These observations indicate that the reason for this high concentration of AM might be secretion of synovial stromal cells or by secretion from synovial vascular wall cells.

However, the mechanism by which plasma and joint fluid AM levels increase in patients with RA remains unknown. Several tissues including vessels secrete AM, and elevated AM could conceivably be caused by secretion from vascular cells in general. When we consider the results, in which AM levels in the synovium of RA patients were higher than those of OA patients, we may assume that synovitis must be one reason for the increased AM concentration in synovium and joint fluid, and may partially contribute to the increase in AM levels in plasma.

Our results indicate the possibility that AM participates in the pathophysiology of joint lesions in patients with RA. Together with the fact that AM is known to inhibit the secretion of cytokines from several cell lines[2,15], the findings seem to validate the assumption that production and secretion of AM in synovium are strongly correlated with antiinflammation in arthritis. Thus, the elevation of plasma AM levels may be related to the antiinflammatory response. Clementi, *et al* investigated the antiinflammatory effect of AM in rats[3], finding that AM production in several cell lines was strongly induced by stimulation of a group of inflammatory cytokines including interleukin 1 and tumor necrosis factor-α[16]. It has also been reported that AM inhibits the secretion of such cytokines in the Swiss 3T3 cell line[2], and some studies reported that AM acted as a circulating vasoactive hormone in blood, and plays an antiinflammatory role in the prevention of local infection and inflammation, thus contributing to host defence systems[4,17]. From these observations, we speculate that AM may play a role in the pathophysiology of inflammation as well as in the regulation of joint disorders.

Synovitis inevitably plays a role in the destruction of joint surface, so we measured the concentration of AM in articular cartilage. We found the AM concentration in cartilage was lower than that of other tissues. While the concentration of AM in normal human articular cartilage has not been determined, an immunohistochemical study has reported that normal human articular chondrocytes produce AM[18]. No statistically significant difference was found in the articular cartilage concentration of AM in RA and OA patients (Figure 5). We therefore speculate that this may be because our samples consisted of endstage arthritis, with advanced degeneration and differentiation of the cartilage. A thorough investigation of the role AM in cartilage pathophysiology is necessary.

We observed that plasma AM concentration in patients with RA is higher than that of healthy controls, and plasma AM and plasma CRP levels were found to be well correlated. Our data suggest that plasma AM levels increase with the activity of RA. We conclude that AM probably plays a part in the regulation of the inflammatory process of RA, and its plasma and/or joint fluid levels could be used as an index of the degree of RA.

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