Angiotensin converting enzyme in human synovium: increased stromal [¹²⁵I]351A binding in rheumatoid arthritis

David Andrew Walsh, John Catravas, John Wharton

Abstract

Objective—To determine whether tissue angiotensin converting enzyme (ACE) is increased in synovia from patients with rheumatoid arthritis, osteoarthritis or chondromalacia patellae.

Methods—Sections of synovia from patients with rheumatoid arthritis (n = 7), osteoarthritis (n = 7) or chondromalacia patellae (n = 6) were tested for immunoreactivity for ACE, and for binding of the ACE inhibitor [¹²⁵I]351A. The amount of ACE was measured with computer assisted image analysis as the proportion of synovial section area occupied by ACE-immunoreactive cells, and the density of [¹²⁵I]351A binding.

Results—[¹²⁵I]351A binding sites had characteristics of ACE and colocalised with ACE-like immunoreactivity to microvascular endothelium and fibroblast-like stromal cells in inflamed and non-inflamed human synovium. Stromal [¹²⁵I]351A binding densities (B_{eq}) and the fraction of synovial section area occupied by ACE-immunoreactivity (fractional area) were higher in synovia from patients with rheumatoid arthritis (B_{eq} 28 amol/mm², fractional area 0.21) than from those with osteoarthritis (B_{eq} 9 amol/mm², fractional area 0.10) or chondromalacia patellae (B_{eq} 9 amol/mm², fractional area 0.09) (p < 0.05). Density of [¹²⁵I]351A binding to stroma was similar to that to blood vessels in rheumatoid arthritis, but less dense than vascular binding in chondromalacia patellae and osteoarthritis. Increases in [¹²⁵I]351A binding densities were attributable to increases in the numbers of binding sites, and were consistent with an increase in the density of ACE bearing stromal cells.

Conclusion—ACE is upregulated in synovial stroma in rheumatoid arthritis. Increased tissue ACE may result in increased local generation of the vasoconstrictor and mitogenic peptide angiotensin II and thereby potentiate synovial hypoxia and proliferation in rheumatoid arthritis.

(Ann Rheum Dis 2000;59:125–131)

Angiotensin converting enzyme (ACE, EC 3.4.15.1) is a membrane metallopeptidase that participates in tissue regulatory peptide systems involving angiotensin II (ANG II), bradykinin and substance P.¹ ACE catalyses the for-

mation of ANG II from its inactive precursor, ANG I, which itself is generated by cleavage of angiotensinogen by the protease renin. ANG II exerts its biological effects via specific, cell surface receptors, of which two major subtypes, named AT₁ and AT₂ receptors, have been identified in humans.²

ANG II is a potent vasoconstrictor, and can stimulate angiogenesis, fibroblast proliferation, collagen synthesis and growth factor expression, each mediated by AT₁ receptors.^{3–7} ACE expression is upregulated by fibroblasts and myofibroblasts after tissue injury.^{8,9} Furthermore, ACE inhibitors and AT₁ receptor antagonists can inhibit interstitial hypertrophy, hyperplasia and angiogenesis in a variety of tissues.^{3,10–13}

Hypertrophy, hyperplasia and angiogenesis also are characteristic of persistent rheumatoid synovitis. ACE activity is raised in synovial fluids from patients with rheumatoid arthritis compared with those with osteoarthritis, and rheumatoid synovial explants release ACE activity into their culture medium indicating local synthesis.^{14,15} ACE, however, is primarily a membrane bound peptidase, and tissue ACE may be more important than soluble ACE in the local generation of ANG II.¹⁶ In human synovium, ACE-like immunoreactivity (ACE-LI) is localised both to vascular endothelium, and to fibroblast-like and some macrophage-like cells in the synovial stroma.¹⁷ It is currently unknown, however, whether tissue ACE is increased in either or both of these synovial compartments in rheumatoid arthritis. Increased tissue ACE could potentiate synovial hypertrophy and hyperplasia in rheumatoid arthritis through the increased generation of ANG II.

[¹²⁵I]351A, an iodotyrosyl derivative of the ACE inhibitor lisinopril, binds specifically to the active site of ACE, and the density of [¹²⁵I]351A binding correlates closely with enzyme activity.^{18,19} Immunohistochemistry is a sensitive technique for localising ACE immunoreactivity and quantifying the proportion of tissue components expressing ACE. Quantitative in vitro receptor autoradiography permits the density of ACE-like binding sites to be measured and pharmacologically characterised. A combination of the two techniques performed on the same tissue samples provides a powerful tool to localise and quantify ACE at the microscopic level in human synovium.

We have now used quantitative in vitro autoradiography with [¹²⁵I]351A and immunohistochemistry to test whether tissue ACE levels are

Academic
Rheumatology,
University of
Nottingham Clinical
Sciences Building,
City Hospital,
Nottingham NG5 1PB
D A Walsh

Vascular Biology
Center, Medical
College of Georgia,
Augusta, Georgia USA
J Catravas

Department of
Histochemistry,
Imperial College
School of Medicine,
Hammersmith
Campus, London
J Wharton

Correspondence to:
Dr Walsh

Accepted for publication
22 October 1999

increased in vascular and stromal synovial compartments in human arthritis.

Methods

PATIENTS

Human synovium was collected at joint arthroplasty from patients with osteoarthritis or rheumatoid arthritis, diagnosed according to American Rheumatism Association criteria,^{20, 21} and from patients undergoing carbon fibre resurfacing of articular cartilage for chondromalacia patellae. Patients with chondromalacia patellae had radiographically normal knees, with cartilage defects observed at arthroscopy.

Equilibrium [¹²⁵I]351A binding studies and ACE immunohistochemistry were performed on synovia from patients with chondromalacia patellae (n = 6, 6 male, median age = 40 years, range 22 to 60), osteoarthritis (n = 7, 4 male, median age = 51 years, range 40 to 70), or rheumatoid arthritis (n = 7, 2 male, median age = 62 years, range 51 to 74). [¹²⁵I]351A saturation studies and assessments of synovial cellularity were performed on synovia from patients with osteoarthritis (n = 7, 5 male, median age = 66 years, range 43 to 78), or rheumatoid arthritis (n = 6, 1 male, median age 61, range 51 to 68).

TISSUE PREPARATION

Tissue samples were embedded in Tissue-Tek (Miles Inc, Elkhart, Indiana, USA) and frozen to cork mounts in melting isopentane without prior fixation. Specimens were stored at -70°C until use. Ten µm thick sections were cut in a cryostat and thaw mounted onto Vectabond (Vector Laboratories, Peterborough, UK) treated slides, air dried and used immediately.

[¹²⁵I]351A BINDING

[¹²⁵I]351A was prepared as previously described by radioiodination of the tyrosyl group of 351A.²² [¹²⁵I]351A was purified to a specific activity of 2000 Ci/mmol by high performance liquid chromatography using a Waters C18 µBondapak analytical column utilising a mobile phase consisting of 88% 0.04 M phosphoric acid with triethylamine, pH 3 and 12% acetonitrile for 10 minutes, followed by a linear gradient from 12% to 25% acetonitrile over the next 40 minutes. The retention time of the monoiodinated product was 24 minutes. [¹²⁵I]351A was stored in the dark at 4°C until use.

Sections of synovium were preincubated for 10 minutes in 10 mM PBS, pH 7.4 (buffer A). Excess buffer was removed and sections were incubated for three hours with 0.03 nM [¹²⁵I]351A in buffer B (buffer A plus 0.2% bovine serum albumin). Non-specific binding was defined in the presence of 1 mM EDTA.¹⁸ After incubations, sections were washed twice for five minutes at 4°C in buffer A and rinsed in distilled water before being rapidly dried under a stream of cold air. Unless otherwise stated, all incubations were performed at 22°C.

QUANTIFICATION OF RADIOLIGAND BINDING

Labelled sections were apposed to radiosensitive film (Hyperfilm-³H, Amersham, UK) and

exposed at 4°C for three to four days, or for five and 20 hours (saturation studies). Films were developed in Kodak D19 for three minutes at 15°C and autoradiographic images were analysed using a Symphony image analysis system (Seescan, Cambridge, UK). Tissue structures were segmented according to grey level, isolated by interactive thresholding then delineated interactively. Vascular [¹²⁵I]351A binding was discriminated from stromal binding in regions of synovium where the density of binding to vessels was greater than that to stroma, resulting in a mask of linear and annular features that corresponded to vessels in sections stained with haematoxylin and eosin or for ACE-like immunoreactivity (see below). Stromal binding was defined as binding to those regions of synovium that were excluded by this vascular mask. Binding density was derived from grey values by comparison with 10 µm thick ¹²⁵Iodine standards (Amersham, UK) coexposed with each film, and corrected for the activity date of the ligand, assuming catastrophe decay.²³ Equilibrium binding densities (B_{eq}) and maximal binding capacities (B_{max}) are expressed as amount of bound ligand (amol) per mm² of blood vessel, or per mm² of stroma.

CHARACTERISATION OF BINDING SITES

Specific binding was defined as total minus non-specific binding. Equilibrium binding studies were performed on triplicate sections. Equilibrium binding density (B_{eq}) was defined as the amount of [¹²⁵I]351A bound per unit area of the structure of interest after equilibration with an excess of 0.03 nM [¹²⁵I]351A. Maximal binding capacities (B_{max}) and equilibrium dissociation constants (K_d) were calculated from saturation studies using 0.05 to 1 nM [¹²⁵I]351A. B_{max} was defined as the total number of binding sites per unit area of the structure of interest. K_d was defined as the concentration of [¹²⁵I]351A leading to 50% occupancy of specific binding sites. Low K_d values indicate high affinity for [¹²⁵I]351A. Observed association rate constants (k_{obs}), dissociation rate constants (k_i) and binding inhibition constants (K_i) were derived from studies performed on synovia from patients with rheumatoid arthritis. High k_{obs} values indicate a rapid rate of association of a given concentration of radioligand with its binding sites. High k_i values indicate rapid dissociation of radioligand from its binding sites. Dissociation was assessed after incubation of sections with 0.03 nM [¹²⁵I]351A for 180 minutes followed by transfer of sections for a further 20 to 120 minutes to an excess of buffer A containing no radioligand. In inhibition studies, sections were incubated with 0.03 nM [¹²⁵I]351A together with 0 to 10 µM non-radiolabelled ligand. Low K_i values indicate that a non-radiolabelled ligand has high affinity for the [¹²⁵I]351A binding site.

IMMUNOHISTOCHEMISTRY

Consecutive sections to those used in binding studies were used for immunolocalisation of ACE using the affinity purified rabbit

Figure 1 Localisation of [¹²⁵I]351A binding sites and angiotensin converting enzyme-like immunoreactivity (ACE-LI) in human synovium. (A) [¹²⁵I]351A binding to synovium from a patient with chondromalacia patellae showing punctate binding to blood vessels (small arrows), with less dense binding to stromal tissue between vessels. (B) ACE-LI in the field of a consecutive tissue section that corresponds to the box shown in (A). Small arrows indicate ACE-LI localised to vascular endothelium corresponding to the punctate [¹²⁵I]351A binding in (A). Less intense ACE-LI is localised to spindle shaped cells within the synovial stroma. (C) [¹²⁵I]351A binding to synovium from a patient with rheumatoid arthritis showing diffuse stromal binding of a similar density to punctate binding (small arrow). (D) ACE-LI in the field of a consecutive tissue section that corresponds to the box shown in (C). Intense ACE-LI is localised to stromal cells, particularly those immediately beneath the lining cells and around blood vessels. The small arrow indicates ACE-LI localised to vascular endothelium corresponding to punctate binding in (C). (A) and (C) Reversal prints of film autoradiograms. (B) and (D) Immunohistochemistry with rabbit polyclonal antihuman ACE, developed with DAB using glucose oxidase/nickel enhancement. Open arrows indicate synovial lining region. Bars = 500 µm.

polyclonal antihuman ACE antibody RH179.²⁴ Cryostat sections (10 µm thick) were stained by the avidin-biotin complex (ABC) method.^{25–26} In brief, sections were fixed in cold acetone for five minutes at 4°C, incubated with primary antibody overnight at 4°C, then with biotinylated goat antirabbit IgG for 30 minutes, avidin-biotin-peroxidase complex for 30 minutes and finally developed in diaminobenzidine with glucose oxidase enhancement, dehydrated and mounted in dibutylphthalate polystyrene xylene (Raymond Lamb, London, UK)

QUANTIFICATION OF IMMUNOHISTOCHEMISTRY AND CELLULARITY

The fraction occupied by ACE immunoreactivity of synovial tissue section area within 200 µm of the synovial surface was measured using a KS300 image analysis system (Imaging Associates, Thame, UK). Using a × 20 objective lens, the three microscopic fields with the most intense stromal ACE immunoreactivity were selected per section. For each field, the synovial area of interest was delineated interactively, and the area occupied by ACE immunoreactivity was segmented according to grey level. ACE fractional area was defined as the sum of the ACE immunoreactive areas divided by the sum of the synovial areas.

Qualitative assessment of inflammation and quantification of cellularity were determined on haematoxylin and eosin counterstained sections used in saturation studies described above. Using a × 10 objective lens, the three

microscopic fields with the most intense cellularity were selected per section. For each field, the synovial area of interest was delineated interactively, and the area occupied by haematoxylin positive nuclei was segmented according to hue. Nuclear fractional area was defined as the sum of the haematoxylin positive areas divided by the sum of the synovial areas.

STATISTICAL ANALYSIS

Descriptive data are expressed as geometric (binding and cellularity data) or arithmetic (ACE fractional area) means (95% confidence intervals). Between group comparisons were made using SPSS for Windows release 6.1 statistical programme (SPSS Inc, Chicago, IL) by Student's *t* test, or one way analysis of variance with post-hoc comparisons using Duncan's multiple range test and a significance level set at 0.05. Correlations were determined using

Table 1 Characteristics of [¹²⁵I]351A binding to human synovium

	Stroma	Blood vessels
<i>Kinetic studies</i>		
<i>K_{obs}</i> (× 10 ⁻³ /s)	14 (7 to 29)	23 (16 to 33)
<i>k₋₁</i> (× 10 ⁻³ /s)	9 (5 to 15)	8 (5 to 15)
<i>K_i values (nM)</i>		
Lisinopril	0.6 (0.2 to 1.4)	0.3 (0.1 to 1.9)
Captopril	1.3 (0.3 to 5.2)	0.7 (0.2 to 2.8)
Phosphoramidon	>10000	>10000

Values are means (95% CI) derived from synovia from six patients with rheumatoid arthritis. *K_{obs}* ; observed association rate constant, *k₋₁* ; dissociation rate constant, *K_i* ; inhibition constant.

Table 2 Density and affinity of [¹²⁵I]351A binding to human synovium

	Stroma			Blood vessels		
	CMP (n=6)	OA (n=7)	RA (n=6-7)	CMP (n=6)	OA (n=7)	RA (n=6-7)
B _{eq} (amol/mm ⁻²)	9 (3 to 25)	9 (4 to 23)	28 (16 to 48)*	64 (46 to 90)	50 (37 to 66)	69 (49 to 97)
B _{max} (amol/mm ⁻²)	NA	118 (61 to 229)	259 (154 to 435)†	NA	380 (188 to 769)‡	360 (144 to 902)
K _d (nM)	NA	0.2 (0.1 to 0.3)	0.3 (0.2 to 0.4)	NA	0.1 (0.1 to 0.2)	0.3 (0.1 to 0.5)

*Significant heterogeneity between B_{eq} values in stroma from patients with CMP, OA and RA (*F*=3.71, *p*=0.04). RA > OA and RA > CMP (*p*<0.05). †B_{max} values for binding to stroma greater in RA than in OA (*t*=2.3, *p*=0.04). ‡B_{max} values greater for binding to blood vessels than to stroma in OA (*t*=6.1, *p*<0.001), but not in RA. No significant differences in K_d values were found between disease groups. Binding to blood vessels did not differ significantly between disease groups. Values are means (95% CI). Abbreviations: B_{eq}; equilibrium binding density, B_{max}; maximal binding capacity, K_d; equilibrium dissociation constant, NA; not assessed, CMP; chondromalacia patellae, OA; osteoarthritis, RA; rheumatoid arthritis.

linear regression. The effect of introducing log₁₀ nuclear fractional area as a covariate on the effect of diagnostic group on maximal binding capacity was determined using a general factorial model. Equilibrium constants were derived from specific binding values by iterative non-linear regression assuming single site models using GraphPAD Prism (San Diego, USA). Values for binding inhibition constants (K_i) were derived by fitting to sigmoid curves assuming competitive interaction.

REAGENTS
351A (N-[(s)-1-carboxy-3-phenylpropyl]-L-lysyl-tyrosyl-L-proline) was provided by Dr M Hichens (Merck, Sharp and Dohme Laboratories, West Point, PA). [¹²⁵I] standards, and Hyperfilm-³H were from Amersham International plc, Amersham, UK. Lisinopril, captopril and phosphoramidon, EDTA and enzyme free bovine serum albumin were from Sigma Chemical Co, Poole, UK. Polyclonal antibodies to human ACE were a kind gift of Dr A J

Kenny, Membrane Peptidase Group, University of Leeds, UK. Biotinylated antibodies and avidin-biotin complexes were from Vector Laboratories, Peterborough, UK.

Results
[¹²⁵I]351A binding sites had an identical distribution to ACE-LI both in inflamed and in non-inflamed synovium (fig 1). ACE-LI was localised to vascular endothelium and to stromal cells (fig 1). As previously described, stromal staining was localised to spindle shaped cells particularly those surrounding blood vessels and those immediately beneath the synovial lining cells.¹⁷ Both ACE-LI and [¹²⁵I]351A binding appeared more dense to stromal cells in synovia from patients with rheumatoid arthritis, compared with osteoarthritis or chondromalacia patellae (fig 1).
[¹²⁵I]351A binding to both vascular and non-vascular structures had characteristics of ACE (tables 1 and 2, fig 2). Binding was time dependent, reversible, saturable, and completely inhibited by the non-radiolabelled ACE

Figure 2 Characteristics of [¹²⁵I]351A binding to human synovium. (A) Association (filled circles) and dissociation (empty circles) at 22°C of 0.03 nM [¹²⁵I]351A binding to blood vessels in synovia from patients with rheumatoid arthritis. For dissociation time course, sections were transferred to an excess of buffer without [¹²⁵I]351A at 180 minutes (arrow). (B) Inhibition of 0.03 nM [¹²⁵I]351A binding to blood vessels in synovia from patients with rheumatoid arthritis by unlabelled lisinopril (circles) or captopril (triangles). (C) Saturation of [¹²⁵I]351A binding to blood vessels in synovia from patients with rheumatoid arthritis. (D) Saturation of [¹²⁵I]351A binding to synovial stroma from patients with rheumatoid arthritis (filled circles) or osteoarthritis (empty circles). Points represent means (SEM) for synovia from six to eight patients.

inhibitors lisinopril and captopril, but not by the neutral endopeptidase inhibitor phosphoramidon (table 1). [125 I]351A binding was inhibited by 97% (95% CI, 93 to 98) in the presence of 1 mM EDTA.

Stromal equilibrium binding of [125 I]351A was higher in synovia from patients with rheumatoid arthritis than from those with osteoarthritis or chondromalacia patellae (table 2). Subsequent saturation studies confirmed the increased [125 I]351A binding to synovial stroma in rheumatoid arthritis, attributable to a twofold increase in the number of binding sites ($B_{\max}$), with no increase in their affinity (indicated by equilibrium dissociation constant (K_d)) (table 2, fig 2). Binding sites on blood vessels were more dense than on stroma in synovia from patients with osteoarthritis, whereas binding sites on blood vessels had a similar density to those in stroma in rheumatoid arthritis (table 2, fig 1).

The fraction of synovial section area occupied by ACE immunoreactivity differed between disease groups ($F = 5.736$, $p = 0.012$). ACE fractional area was greater in synovia from patients with rheumatoid arthritis (0.21 (95% CI, 0.14 to 0.28)), than in synovia from patients with osteoarthritis (0.10 (95% CI, 0.03 to 0.18), $p < 0.05$), and synovia from patients with chondromalacia patellae (0.09 (95% CI, 0.05 to 0.14), $p < 0.05$). ACE fractional area displayed positive correlation with $\log_{10}$ stromal [125 I]351A equilibrium binding density ($r^2 = 0.41$, $F = 15.3$, $p = 0.001$).

Four of six synovial samples from patients with rheumatoid arthritis, and two of seven samples from patients with osteoarthritis displayed perivascular accumulations of lymphocytes. The fraction of synovial section area occupied by haematoxylin positive nuclei was greater in samples from patients with rheumatoid arthritis (0.07 (95% CI, 0.05 to 0.10)), than in samples from patients with osteoarthritis (0.03 (95% CI, 0.02 to 0.06), $p = 0.02$). $\log_{10}$ stromal [125 I]351A maximal binding capacity displayed positive correlation with $\log_{10}$ nuclear fractional area ($r^2 = 0.32$, $F = 5.12$, $p = 0.04$). Introduction of $\log_{10}$ nuclear fractional area as a covariate resulted in no significant difference in $\log_{10}$ stromal [125 I]351A maximal binding capacity being displayed between samples from patients with rheumatoid arthritis and those with osteoarthritis ($F = 1.12$, $p = 0.32$).

Discussion

Our findings indicate that tissue ACE levels are increased in synovia from patients with rheumatoid arthritis and that this increase is attributable to an increase in the density of ACE bearing stromal cells, rather than to changes in vascular ACE.

The distribution of [125 I]351A binding was identical to that of ACE-LI, confirming the localisation of ACE to synovial vascular endothelium, and to fibroblast-like stromal cells, particularly those localised immediately beneath the synovial lining cells.¹⁷ Stromal [125 I]351A binding, as defined in this study, included some binding to vascular endothe-

lium, where vessels were small or displayed low density binding that did not allow their discrimination from stroma in the vascular mask. Differences in vascular densities between disease groups are unlikely to explain differences in stromal [125 I]351A binding because two parallel studies using immunoreactivity for CD34 and binding of *Ulex europaeus* agglutinin-I as endothelial markers did not indicate differences in the fraction of synovial section area occupied by endothelium between these disease groups.²⁷ Furthermore, the endothelial fractional areas (approximately 8%) determined in each of three disease groups in these studies were similar to ACE fractional areas in synovia from patients with chondromalacia patellae or osteoarthritis in this study, and less than half the values for ACE fractional areas in synovia from patients with rheumatoid arthritis. Qualitative analysis of synovial sections stained for ACE-like immunoreactivity further supported our interpretation that increased stromal [125 I]351A binding in rheumatoid arthritis reflects increased ACE on non-vascular cells (fig 1).

[125 I]351A binding was saturable, of high affinity and reversible. It was completely inhibited by the zinc chelator EDTA, and by the specific ACE inhibitors lisinopril and captopril, but not by the neutral endopeptidase inhibitor phosphoramidon at up to 10 μ M. These characteristics of [125 I]351A binding sites both on blood vessels and on stroma in human synovia were consistent with binding to the active site of ACE, which has previously been correlated with ACE activity.^{18,19} These findings indicate that the increased stromal [125 I]351A binding observed in synovia from patients with rheumatoid arthritis represents increased amounts of ACE.

Increased stromal ACE may indicate either an increase density of cells that express ACE, or an increase in ACE expression by stromal cells. Either of these interpretations of our data would be consistent with increased ACE activity in the synovia of patients with rheumatoid arthritis that may contribute to the raised ACE activity observed in synovial fluids from patients with rheumatoid arthritis.^{14,15} Cellularity was increased in synovia from patients with rheumatoid arthritis compared with those from patients with osteoarthritis, as indicated by an increased proportion of the section area occupied by haematoxylin positive nuclei. This increase in cellularity was sufficient to explain the increased [125 I]351A binding capacity in rheumatoid arthritis, supporting the suggestion that increased stromal ACE reflects an increased number of ACE bearing cells within the synovium. Clarification of whether individual cells express more ACE in synovia from patients with rheumatoid arthritis will require further work on primary cultures of synovial cells.

Increased ACE in tissue stroma has been observed in the heart after myocardial infarction and in hypertension, and in healing skin wounds.^{8,9,28} As in synovium, stromal ACE was localised to fibroblast-like cells in these tissues. Cultured fibroblasts and myofibroblasts

express ACE mRNA and protein, and display ACE-like enzymatic activity.^{5 29 30} ACE expression by fibroblast-like cells is generally lower than by endothelial cells.^{31 32} However, fibroblast expression of ACE is subject to upregulation by a variety of factors that are present in inflamed synovium, such as basic fibroblast growth factor.⁵

Serum concentrations of angiotensinogen, the precursor of ANG I, are increased, and synovial fluid renin activity is raised in rheumatoid arthritis.^{33 34} Increased concentrations both of ANG II precursors and of ACE lead us to suggest that the synovial angiotensin system is activated in rheumatoid arthritis. Furthermore, AT₁ receptor binding and ACE colocalise in human synovium indicating that synovial AT₁ receptors are exposed to locally generated ANG II.³⁵

ANG II that has been generated by stromal cells may have autocrine effects on fibroblasts. Synovial inflammation may be regarded as an abnormal repair process in which synovial proliferation leads to joint damage. ANG II, acting through AT₁ receptors, can stimulate fibroblast proliferation and the generation of matrix proteins and growth factors such as transforming growth factor β .^{4 6 7} ACE is upregulated on cardiac fibroblasts during the tissue repair that follows myocardial infarction.⁹ In this model, fibroblast proliferation is inhibited by specific ACE inhibitors and by AT₁ receptor antagonists, consistent with stimulatory roles for endogenous ACE and ANG II.^{11 12} Increased stromal ACE may contribute to synovial proliferation and hypertrophy in rheumatoid arthritis by increasing ANG II production within the stromal compartment.

ANG II produced by stromal cells may have paracrine actions on the synovial vasculature, in addition to autocrine effects on synovial fibroblast-like cells. ANG II is a potent vasoconstrictor, and can stimulate angiogenesis in inflamed tissues.³ Increased ANG II generation within inflamed synovium therefore may contribute to synovial hypoxia and angiogenesis, each of which has been implicated in the pathogenesis of persistent synovitis.^{27 36}

In addition to its role in the renin-angiotensin system, ACE can inactivate other peptides including bradykinin and substance P.¹ These peptides increase blood flow and plasma extravasation in acute models of inflammation, and may stimulate vascular and connective tissue proliferation in chronic inflammation.³⁷⁻³⁹ Synovial stromal cells and microvascular endothelium bear specific receptors for bradykinin and substance P respectively^{40 41} and upregulation of synovial ACE may be important in reducing the pro-inflammatory activities of these peptides.

The ACE inhibitor captopril had effects that were consistent with a slow acting anti-rheumatic agent in an open, prospective study of 15 patients with rheumatoid arthritis.⁴² ACE inhibition with pentopril, however, was not associated with clinical improvement in rheumatoid arthritis.⁴³ These equivocal results may reflect the different roles of ACE in multiple regulatory peptide systems. In animal models,

effects of ACE inhibition have sometimes been attributed to potentiation of kinins or substance P, rather than inhibition of ANG II generation.^{44 45} Elucidation of the importance of increased synovial ANG II generation in rheumatoid arthritis will require more selective intervention with agents such as the recently developed AT₁ receptor antagonists.²

- Kenny AJ, Stephenson SL, Turner AJ. Cell surface peptidases. In: Kenny AJ, Turner AJ, eds. *Mammalian ectoenzymes*. Oxford: Elsevier, 1987:169-210.
- Timmermans PB, Wong PC, Chiu AT, Herblin WF, Benfield P, Carini DJ, et al. Angiotensin II receptors and angiotensin II receptor antagonists. *Pharmacol Rev* 1993; 45:205-51.
- Walsh DA, Hu DE, Wharton J, Catravas JD, Blake DR, Fan TP. Sequential development of angiotensin receptors and angiotensin I converting enzyme during angiogenesis in the rat subcutaneous sponge granuloma. *Br J Pharmacol* 1997; 120:1302-11.
- Brilla CG, Zhou G, Matsubara L, Weber KT. Collagen metabolism in cultured adult rat cardiac fibroblasts: response to angiotensin II and aldosterone. *J Mol Cell Cardiol* 1994;26:809-20.
- Hafizi S, Wharton J, Morgan K, Allen SP, Chester AH, Catravas JD, et al. Expression of functional angiotensin-converting enzyme and AT₁ receptors in cultured human cardiac fibroblasts. *Circulation* 1998;98:2553-9.
- Sadoshima J, Izumo S. Molecular characterization of angiotensin II-induced hypertrophy of cardiac myocytes and hyperplasia of cardiac fibroblasts. Critical role of the AT₁ receptor subtype. *Circ Res* 1993;73:413-23.
- Fisher SA, Absher M. Norepinephrine and ANG II stimulate secretion of TGF-beta by neonatal rat cardiac fibroblasts in vitro. *Am J Physiol* 1995;268:C910-17.
- Sun Y, Weber KT. Angiotensin-converting enzyme and wound healing in diverse tissues of the rat. *J Lab Clin Med* 1996;127:94-101.
- Sun Y, Cleutjens JP, Diaz-Arias AA, Weber KT. Cardiac angiotensin converting enzyme and myocardial fibrosis in the rat. *Cardiovasc Res* 1994;28:1423-32.
- Wu LL, Yang N, Roe CJ, Cooper ME, Gilbert RE, Atkins RC, et al. Macrophage and myofibroblast proliferation in remnant kidney: role of angiotensin II. *Kidney Int* 1997;63:S221-5.
- Smits JF, van Krimpen C, Schoemaker RG, Cleutjens JP, Daemen MJ. Angiotensin II receptor blockade after myocardial infarction in rats: effects on hemodynamics, myocardial DNA synthesis, and interstitial collagen content. *J Cardiovasc Pharmacol* 1992;20:772-8.
- van Krimpen C, Smits JF, Cleutjens JP, Debets JJ, Schoemaker RG, Struyker Boudier HA, et al. DNA synthesis in the non-infarcted cardiac interstitium after left coronary artery ligation in the rat: effects of captopril. *J Mol Cell Cardiol* 1991;23:1245-53.
- Volpert OV, Ward WF, Lingen MW, Chesler L, Solt DB, Johnson MD, et al. Captopril inhibits angiogenesis and slows the growth of experimental tumors in rats. *J Clin Invest* 1996;98:671-9.
- Lowe JR, Dixon JS, Guthrie JA, McWhinney P. Serum and synovial fluid levels of angiotensin converting enzyme in polyarthritis. *Ann Rheum Dis* 1986;45:921-4.
- Veale D, Yanni G, Bresnahan B, FitzGerald O. Production of angiotensin converting enzyme by rheumatoid synovial membrane. *Ann Rheum Dis* 1992;51:476-80.
- Unger T, Gohlke P. Tissue renin-angiotensin systems in the heart and vasculature: possible involvement in the cardiovascular actions of converting enzyme inhibitors. *Am J Cardiol* 1990;65:3-101.
- Walsh DA, Mapp PI, Wharton J, Polak JM, Blake DR. Neuropeptide degrading enzymes in normal and inflamed human synovium. *Am J Pathol* 1993;142:1610-21.
- Mendelsohn FA. Localization of angiotensin converting enzyme in rat forebrain and other tissues by in vitro autoradiography using 125I-labelled MK351A. *Clin Exp Pharmacol Physiol* 1984;11:431-5.
- Correa FM, Guilhaume SS, Saavedra JM. Comparative quantification of rat brain and pituitary angiotensin-converting enzyme with autoradiographic and enzymatic methods. *Brain Res* 1991;545:215-22.
- Altman R, Asch E, Bloch D, Bole G, Borenstein D, Brandt K, et al. Development of criteria for the classification and reporting of osteoarthritis. Classification of osteoarthritis of the knee. Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association. *Arthritis Rheum* 1986;29:1039-49.
- Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315-24.
- Fyhrquist F, Tikkanen I, Gronhagen-Riska C, Hortling L, Hichens M. Inhibitor binding assay for angiotensin-converting enzyme. *Clin Chem* 1984;30:696-700.
- Catt KJ, Baukal A. Prolonged retention of high specific activity by 125I-labeled angiotensin II—a consequence of 'decay catastrophe'. *Biochim Biophys Acta* 1973;313:221-5.

- 24 Howell S, Brewis IA, Hooper NM, Kenny AJ, Turner AJ. Mosaic expression of membrane peptidases by confluent cultures of Caco-2 cells. *FEBS Lett* 1993;317:109–12.
- 25 Hsu SM, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem* 1981; 29:577–80.
- 26 Shu SY, Ju G, Fan LZ. The glucose oxidase-DAB-nickel method in peroxidase histochemistry of the nervous system. *Neurosci Lett* 1988;85:169–71.
- 27 Walsh DA, Wade M, Mapp PI, Blake DR. Focally regulated endothelial proliferation and cell death in human synovium. *Am J Pathol* 1998;152:691–702.
- 28 Challah M, Nicoletti A, Arnal JF, Philippe M, Laboulandine I, Allegrini J, *et al.* Cardiac angiotensin converting enzyme overproduction indicates interstitial activation in renovascular hypertension. *Cardiovasc Res* 1995;30:231–9.
- 29 Weinberg KS, Douglas WH, MacNamee DR, Lanzillo JJ, Fanburg BL. Angiotensin I-converting enzyme localization on cultured fibroblasts by immunofluorescence. *In Vitro* 1982;18:400–6.
- 30 Katwa LC, Campbell SE, Tyagi SC, Lee SJ, Cicila GT, Weber KT. Cultured myofibroblasts generate angiotensin peptides de novo. *J Mol Cell Cardiol* 1997;29:1375–86.
- 31 Rubin DB, Mason RJ, Dobbs LJ. Angiotensin-converting enzyme substrates hydrolyzed by fibroblasts and vascular endothelial cells. *Exp Lung Res* 1982;3:137–45.
- 32 Wharton J, Morgan K, Rutherford RA, Catravas JD, Chester A, Whitehead BF, *et al.* Differential distribution of angiotensin AT2 receptors in the normal and failing human heart. *J Pharmacol Exp Ther* 1998;284:323–36.
- 33 Metsarinne KP, Nordstrom DC, Kontinen YT, Teppo AM, Fyhrquist FY. Plasma interleukin-6 and renin substrate in reactive arthritis, rheumatoid arthritis, and systemic lupus erythematosus. *Rheumatol Int* 1992;12:93–6.
- 34 Izai M, Miyazaki S, Murai R, Morioka Y, Hayashi H, Nishiura M, *et al.* Prorenin-renin axis in synovial fluid in patients with rheumatoid arthritis and osteoarthritis. *Endocrinol Jpn* 1992;39:259–67.
- 35 Walsh DA, Suzuki T, Knock GA, Blake DR, Polak JM, Wharton J. AT1 receptor characteristics of angiotensin analogue binding in human synovium. *Br J Pharmacol* 1994;112:435–42.
- 36 Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat Med* 1995;1:27–31.
- 37 Walsh DA, Fan T-PD. Bradykinin as a growth factor. In: Farmer SG, ed. *The kinin system*. London: Academic Press, 1997:301–14.
- 38 Fan TP, Hu DE, Guard S, Gresham GA, Watling KJ. Stimulation of angiogenesis by substance P and interleukin-1 in the rat and its inhibition by NK1 or interleukin-1 receptor antagonists. *Br J Pharmacol* 1993; 110:43–9.
- 39 Nilsson J, von Euler AM, Dalsgaard CJ. Stimulation of connective tissue cell growth by substance P and substance K. *Nature* 1985;315:61–3.
- 40 Walsh DA, Salmon M, Mapp PI, Wharton J, Garrett N, Blake DR, *et al.* Microvascular substance P binding to normal and inflamed rat and human synovium. *J Pharmacol Exp Ther* 1993;267:951–60.
- 41 Bathon JM, Manning DC, Goldman DW, Towns MC, Proud D. Characterization of kinin receptors on human synovial cells and upregulation of receptor number by interleukin-1. *J Pharmacol Exp Ther* 1992;260:384–92.
- 42 Martin MF, Surrall KE, McKenna F, Dixon JS, Bird HA, Wright V. Captopril: a new treatment for rheumatoid arthritis? *Lancet* 1984;i:1325–8.
- 43 Bird HA, Le Gallez P, Dixon JS, Catalano MA, Traficante A, Liauw LA, *et al.* A clinical and biochemical assessment of a nonthiol ACE inhibitor (pentopril; CGS-13945) in active rheumatoid arthritis. *J Rheumatol* 1990;17:603–8.
- 44 Caspritz G, Alpermann HG, Schleyerbach R. Influence of the new angiotensin converting enzyme inhibitor ramipril on several models of acute inflammation and the adjuvant arthritis in the rat. *Arzneimittelforschung* 1986;36:1605–8.
- 45 Subissi A, Guelfi M, Criscuolo M. Angiotensin converting enzyme inhibitors potentiate the bronchoconstriction induced by substance P in the guinea-pig. *Br J Pharmacol* 1990;100:502–6.