CONCISE REPORT

Decreased density of serotonin 5-HT2$_A$ receptors in rheumatoid arthritis

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Background: Animal studies have indicated that 5-HT2$_A$ receptors could play a role in arthritic diseases.
Objective: To analyse the binding properties of 5-HT2$_A$ receptors in patients with rheumatoid arthritis.
Methods: Using a radioactive binding assay, 43 patients with rheumatoid arthritis were compared with 49 sex and age matched controls for density and affinity (measured as B_{max} and K_d) of 5-HT2$_A$ serotonin receptors. Genotyping, using polymerase chain reaction, was undertaken to exclude the possibility that differences in the genetic polymorphism T102C for the 5-HT2$_A$ receptor determine differences in receptor density.
Results: Mean of B_{max} of 5-HT2$_A$ receptors in rheumatoid patients was significantly lower than in controls, at 45.3 *v* 57.4 fmol/mg protein ($p = 0.004$), but there was no significant difference in K_d. The T102C receptor polymorphism genotypes showed a skewed distribution between the two groups. Even when adjusted for this, there was a significant difference in B_{max} between the groups.
Conclusions: The density of 5-HT2$_A$ serotonin receptors in patients with rheumatoid arthritis is markedly reduced. This could either reflect a difference involved in the susceptibility to the disease or be a secondary effect of the disease.

Serotonin (5-HT), an amino acid and transmitter substance, has a widespread distribution in the human body. Seven main types of serotonin receptor and several subtypes have been identified. Apart from being found in neurones, receptor subtype 5-HT2$_A$ is present in platelets, vascular smooth muscle, and uterine smooth muscle.[1] Evidence suggests that 5-HT2$_A$ receptor density and affinity (B_{max} and K_d, respectively) in platelets could reflect the receptor binding in other tissues.[2] Presently, at least six polymorphisms have been characterised in the genes for the 5-HT2$_A$ receptor. In position 102 there is a single nucleotide mutation that has been shown to influence receptor binding, with the TT genotype associated with significantly higher B_{max}.[3] There are results from genetic association studies which indicate that 5-HT2$_A$ receptors may be involved in the pathophysiology of fibromyalgia.[4]

In animal models, serotonin has been considered to have a proinflammatory role. When *p*-chlorophenylalanine, a reversible inhibitor of tryptophan hydroxylase which is the rate limiting enzyme of serotonin synthesis, is given to rats with adjuvant induced arthritis (AA), the inflammation is reduced significantly by 30–40%.[5] When 5′7′-dihydroxytryptamine, a serotonin neurotoxin, was injected into the lateral ventricle of the brain of AA rats, an even greater reduction in the severity of the disease was observed.[6] Furthermore, ketanserin, a known blocker of 5-HT2$_A$ receptors, has been shown to reduce acute synovial inflammation in rat knee joints.[7]

This study was carried out to investigate the 5-HT2$_A$ receptor status in patients with rheumatoid arthritis. To exclude the possibility that a skewed distribution of the more uncommon TT genotype in the T102C polymorphism could have influenced the mean values of B_{max} for the 5-HT2$_A$ receptor we carried out genotyping to allow adjustment for the T102C genotype distribution.

METHODS

Patients and controls

Forty three patients (27 women and 16 men) with rheumatoid arthritis were compared with 49 controls (30 women and 19 men). Patients with rheumatoid arthritis, who fulfilled the 1987 American College of Rheumatology criteria,[8] were consecutively recruited to the study at the Department of Rheumatology, University Hospital, Umeå. Clinical characteristics of the patients are presented in table 1. Controls were matched with respect to age and sex, and were recruited from the population register from the same geographic region with help from Statistics Sweden (SCB). The mean age for both patients and controls was 65 years.

The accumulated disease activity score was calculated retrospectively on the basis of erythrocyte sedimentation rate (ESR), the number of swollen and tender joints, and the clinician's global assessment of disease activity for every second year following disease onset.[9]

Based on previous research, factors that could possibly alter 5-HT2$_A$ receptor status were considered as exclusion criteria. For both groups, the presence of known mood disorders and treatment with oral corticosteroids or psychotropic drugs[1] for less than three months before blood sampling were exclusion criteria. As the menstrual cycle can modulate 5-HT2$_A$ receptor status,[2] all participating women had to be postmenopausal, and not treated with high dose oestrogen preparations. It has also been shown that B_{max} is significantly lower in the lighter half of the year than in the darker half, and that K_d is significantly lower in the autumn than in the summer or winter.[2] Therefore, to eliminate the risk that circadian rhythm and light exposure could have influenced the results of our study, all blood samples were drawn under similar seasonal conditions. Furthermore visits to sunny regions with considerably more daylight than in Sweden and the use of a solarium less than three months before blood sampling were also exclusion criteria. For the control group, confirmed or suspected arthritic disease and confirmed arthritis in first degree relatives were also exclusion criteria.

The study was approved by the Regional Research Ethics Committee, University of Umeå, Sweden.

Abbreviations: AA, adjuvant induced arthritis; [^{3}H]LSD, [^{3}H]-lysergic acid diethylamide; 5-HT, 5-hydroxytryptamine (serotonin)

Table 1 Demographic and clinical characteristics of patients with rheumatoid arthritis at the time of the study

Age at inclusion (years)	65.1 (8.0)
Duration of disease (years)	16.8 (12.9)
Age at disease onset (years)	47.4 (14.8)
Accumulated disease activity score*	4.3 (0.8)
DMARDs†	36 (84%)
NSAID, ASA, or paracetamol	23 (53%)
TNFα blockers	2 (5%)

Values are mean (SD) or n (%).
*Calculated according to Baecklund *et al*.[9]
†DMARDS used were methotrexate (n = 30), gold salts (n = 9), ciclosporine A (n = 4), sulfasalazine (n = 3), azathioprine (n = 1), and chloroquine (n = 1).
ASA, aminosalicylic acid; DMARD, disease modifying antirheumatic drug; NSAID, non-steroidal anti-inflammatory drug; TNFα, tumour necrosis factor α.

Blood sampling

Blood was obtained from the antecubital vein for [^{3}H]-lysergic acid diethylamide ([^{3}H]LSD) binding and for genotyping. ESR was measured in both rheumatoid arthritis patients and controls on the day of sampling blood, but for four of the patients and three of the controls the ESR values from the current day were not available.

[^{3}H]LSD binding assay

The method used for platelet [^{3}H]LSD binding assay has been described in detail previously.[2] In short, a platelet pellet was gently homogenised and centrifuged. The resulting membrane pellet was resuspended and incubated with seven concentrations of [^{3}H]LSD. Specific binding was defined as total binding minus binding in the presence of spiperone. All assays were done in triplicate. The binding was terminated by filtration through GF/C filters, using a cell harvester. The radioactivity trapped by the filter was determined by liquid scintillation spectroscopy.

Genotyping

Genomic DNA was extracted from peripheral white blood cells. Primers were synthesised by Scandinavian Gene Synthesis (SGS, Köping, Sweden). The following sequence was used: 5′-TCG GCA TAA CCA ACA AAA TGA GATA-3′ and 5′-CCT GGA GAT GAA GTA AGG AGA GACA-3′ at an annealing temperature of 60°C. The amplified fragments were digested with MSP I and visualised by silver staining in a polyacrylamide gel or by ethidium bromide in an agarose gel.

Statistical analysis

A two sample independent *t* test was used to compare B_{max} and K_d values between groups. Analysis of variance was used to control for sex and genotype. Genotype and allele frequencies were compared using the χ^2 test. Association of B_{max} with duration of disease, age at onset of rheumatoid arthritis, ESR, and accumulated disease activity score, respectively, was tested by Pearson correlation coefficient. Probability (p) values below 0.05 were considered as significant. SPSS software, version 12.0, was used for the statistical analyses.

RESULTS

Density and affinity of 5-HT2$_A$ receptors

The mean values of B_{max} and K_d for [^{3}H]LSD binding to 5-HT2$_A$ receptors in the patients and the controls are presented in table 2. B_{max} was significantly lower ($p = 0.004$) in the rheumatoid arthritis group than in the control group, but there was no significant difference in K_d between the two groups ($p = 0.67$). The significant difference in B_{max} persisted after adjustment for sex and differences in T102C genotype distribution. The difference in B_{max} between the two groups was evident for both men and women, but reached significance only for women ($p = 0.006$). There was no significant difference in B_{max} between patients who were treated with methotrexate, gold salts, all disease modifying antirheumatic drugs considered as one group, or non-steroidal anti-inflammatory drugs/aminosalicylic acid/paracetamol as one group, compared with patients not treated with these drugs (table 2). Both accumulated disease activity score and ESR on the day of sampling correlated inversely with B_{max} ($r = -0.33$, $p = 0.03$, and $r = -0.36$, $p = 0.02$, respectively) in the patients with rheumatoid arthritis, whereas ESR did not correlate with B_{max} ($r = 0.008$; $p = 0.96$) in the controls. Neither disease duration nor age at onset of rheumatoid arthritis correlated significantly with B_{max}.

Genotyping of the T102C polymorphism

The genotype distribution in both groups was in Hardy–Weinberg equilibrium. The genotype distribution differed significantly between the rheumatoid patients and the controls ($\chi^2 = 6.38$, 2 df, $p = 0.04$). The T allele was significantly less frequent in the rheumatoid patient group

Table 2 5-HT2$_A$ receptor density (B_{max}) and affinity (K_d) for patients with rheumatoid arthritis and controls, and for subgroups of rheumatoid arthritis patients based on drugs administered

Group	B_{max} (fmol/mg protein)	p Value	K_d (nmol/l)	p Value
All RA patients	45.3 (17.0)	0.004*	0.83 (0.42)	NS*
Men	47.5 (20.5)	0.25*	0.67 (0.38)	NS*
Women	43.9 (14.8)	0.006*	0.92 (0.41)	NS*
All controls	57.4 (21.8)		0.79 (0.48)	
Men	55.2 (18.2)		0.60 (0.28)	
Women	58.8 (23.9)		0.91 (0.54)	
Treatment				
DMARDs	46.5 (17.5)	NS†	0.84 (0.43)	NS†
Methotrexate	44.2 (17.5)	NS†	0.86 (0.45)	NS†
Gold salts	44.4 (15.2)	NS†	0.80 (0.41)	NS†
NSAID/ASA/paracetamol	45.3 (17.1)	NS†	0.87 (0.34)	NS†

Values are mean (SD).
*Versus controls.
†Versus those patients with rheumatoid arthritis not receiving the drug in question.
ASA, aminosalicylic acid; DMARD, disease modifying antirheumatic drug; NSAID, non-steroidal anti-inflammatory drug; RA, rheumatoid arthritis.

Table 3 Distribution of genotypes for the 5-HT2$_A$ receptor polymorphism T102C, and mean of 5-HT2$_A$ receptor density (B_{max}) divided into groups with different genotypes for rheumatoid arthritis patients and controls, respectively

	RA patients (n = 43)		Controls (n = 49)	
	Genotype distribution	B_{max}†	Genotype distribution	B_{max}†
Genotypes*				
CC	23 (53.5%)	47.4 (18.8)	19 (38.8)	58.6 (19.3)
TC	20 (46.5%)	42.8 (14.8)	24 (49.0)	56.0 (24.9)
TT	0 (0)	–	6 (12.2)	65.0 (16.8)
Alleles**				
C	66 (76.7)		62 (63.3)	
T	20 (23.3)		36 (36.7)	

Values are n (%) or †mean (SD).
*p = 0.04 for genotype distribution rheumatoid arthritis *v* controls.
**p = 0.05 for alleles rheumatoid arthritis *v* controls.
RA, rheumatoid arthritis.

than in the controls, and there was no TT genotype in the rheumatoid group (table 3).

DISCUSSION

In the present study, the density of serotonin 5-HT2$_A$ receptors was considerably lower in our population of rheumatoid patients than in the controls. Additionally, there seemed to be a correlation between more severe disease and lower 5-HT2$_A$ receptor density. The lower B_{max} values could either represent a downregulation of 5-HT2$_A$ receptors secondary to the rheumatic disease or be constitutional, thereby possibly contributing to an increased susceptibility to rheumatoid arthritis. As animal models have shown that stimulation of 5-HT2$_A$ receptors can mediate synovitis[7] a reactive downregulation could reflect or contribute to a compensatory mechanism directed against stimulation of 5-HT2$_A$ receptors involved in the pathophysiology of the inflammation. On the other hand, our study showed differences in the distribution of 5-HT2$_A$ receptor T102C genotypes between the rheumatoid patients and the controls. A previous study has shown an association between the TT genotype and higher B_{max} values.[3] The TT genotype was only present in the control group in our study, and there was a tendency, although not significant, towards higher B_{max} values among those individuals. However, even when adjusting for these differences in genotype distribution, B_{max} was significantly lower in the rheumatoid arthritis group. This lower B_{max} in the rheumatoid group could still be genetically determined, as there are other polymorphisms that might influence the B_{max} of 5-HT2$_A$ receptors. Although a study of genetic polymorphisms for the 5-HT2$_A$ receptor in relation to rheumatoid arthritis was not the primary objective of this study, we interpret the results of the secondary analyses to be sufficiently encouraging to warrant larger genetic studies to establish the role of the T102C polymorphism in rheumatoid arthritis.

A further explanation for the lower B_{max} in the rheumatoid arthritis group could be that a downregulation of 5-HT2$_A$ receptors is induced by any of the drugs used in the rheumatoid arthritis group. Studies in animals have shown that B_{max} for 5-HT2$_A$ receptors can be altered by paracetamol and non-steroidal anti-inflammatory drugs such as acetylsalicylic acid.[10 11] However, this was not found in our study.

Both the 5-HT2$_A$ receptor and the T102C polymorphism have implications for the pathophysiology of schizophrenia.[12 13] In connection with this, it is noteworthy that there is a negative association between schizophrenia and rheumatoid arthritis.[14 15]

The results of our study raise several further questions. Are the changes restricted to platelets or does the 5-HT2$_A$ receptor status in platelets reflect changes in some other type of cells or tissue which have importance for the pathophysiology of rheumatoid arthritis? Are the changes in 5-HT2$_A$ receptor B_{max} restricted to rheumatoid arthritis or could they also be seen in other rheumatic or autoimmune diseases? All such questions have to be addressed in further studies.

In conclusion, we have identified an association between patients with rheumatoid arthritis and a low density of serotonin 5-HT2$_A$ receptors.

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