



5-HT_{2A} – a serotonin receptor with a possible role in joint diseases

Anders Kling

5-HT_{2A} – a serotonin receptor with a possible role in joint diseases

Anders Kling



**Institutionen för farmakologi och klinisk neurovetenskap, Klinisk
farmakologi/ Department of Pharmacology and Clinical
Neuroscience, Clinical Pharmacology**
Umeå universitet/ Umeå University
Umeå 2013

Responsible publisher under swedish law: the Dean of the Medical Faculty
This work is protected by the Swedish Copyright Legislation (Act 1960:729)

ISBN: 978-91-7459-549-9

ISSN: 0346-6612

New series No: 1547

Elektronisk version tillgänglig på <http://umu.diva-portal.org/>

Tryck/Printed by: Print och Media, Umeå universitet

Umeå, Sweden 2013

Innehåll/Table of Contents

Innehåll/Table of Contents	i
Abstract	iv
Abbreviations	vi
List of studies	viii
Populärvetenskaplig sammanfattning	ix
5-HT _{2A} – en serotoninreceptor med möjlig betydelse för ledsjukdomar	ix
Introduction	1
The serotonin system	1
<i>Serotonin</i>	1
<i>Serotonin receptors</i>	2
<i>The serotonin system and platelets</i>	2
Serotonin receptor 5-HT _{2A}	3
<i>Localisation/expression of 5-HT_{2A} receptors</i>	3
<i>Functions of the 5-HT_{2A} receptor</i>	4
<i>Regulation of the 5-HT_{2A} receptor</i>	4
<i>Connections between the 5-HT_{2A} receptor and the HPA-axis</i>	5
<i>HTR2A – The gene encoding for the serotonin receptor 5-HT_{2A}</i>	7
<i>Methods to analyse 5-HT_{2A} receptors</i>	8
<i>Radioligand methods</i>	8
<i>Methods based on genetic analysis</i>	9
<i>Measurements of ion flows</i>	9
<i>Other methods</i>	9
<i>The 5-HT_{2A} receptor and psychiatric diseases</i>	10
Rheumatoid arthritis	11
<i>Rheumatoid arthritis and schizophrenia</i>	12
Serotonin, 5HT _{2A} receptors and inflammatory joint disease	12
Polymyalgia rheumatica and Giant cell arteritis	13
Adverse drug reactions	14
Antidepressant drugs	14
Aims	16
Subjects and Methods	17
Subjects	17
<i>Patient group and control group in study I</i>	17
<i>Patient group and control group in study II</i>	17
<i>PMR cohort used for the study of effects on 5-HT_{2A} receptors caused by medication with glucocorticoids in study IV</i>	18
Ethical approvals	19
Methods	19
<i>[³H]LSD ligand binding of platelets in study I and IV</i>	19
<i>Choice of ligands</i>	19

<i>Additional description concerning the [3H]LSD binding experiments</i>	20
<i>Rules for calculations of B_{max} and K_d for [3H]LSD ligand binding</i>	20
<i>[3H]LSD ligand binding of lymphocytes</i>	21
<i>Genotyping in study I and II</i>	21
<i>Other experimental methods used</i>	22
<i>Epidemiological method in study III</i>	23
<i>Statistical analyses</i>	25
<i>Paper I</i>	25
<i>Paper II</i>	26
<i>Paper III</i>	26
<i>Paper IV</i>	26
<i>General</i>	26
Results	27
<i>5-HT_{2A} receptor density and affinity (study I and IV)</i>	27
<i>5-HT_{2A} receptor density and affinity in RA (study I)</i>	27
<i>5-HT_{2A} receptor density and affinity following glucocorticoid treatment (study IV)</i>	27
<i>Genetic HTR2A association studies (study I and II)</i>	30
<i>Joint symptoms as adverse drug reaction of 5-HT_{2A} antagonists (study III)</i>	31
<i>Joint ADRs in relation to all ADRs</i>	31
<i>Joint ADRs in relation to DDD</i>	31
Discussion	33
<i>Low 5-HT_{2A} receptor density in joint disease – cause or consequence?</i>	33
<i>Other types of receptors and pathways that may influence 5-HT_{2A} density</i>	34
<i>Other diseases or conditions that may have significance for the relation between 5-HT_{2A} receptor density and RA</i>	35
<i>5-HT_{2A} receptors, psychiatric diseases and RA</i>	35
<i>5-HT_{2A} receptors, fibromyalgia and RA</i>	36
<i>5-HT_{2A} receptors, cardiovascular disease and RA</i>	36
<i>5-HT_{2A} receptors, cigarette smoking and RA</i>	36
<i>Possible mechanisms behind the results</i>	37
<i>Are the 5-HT_{2A} receptors on neurons of significance in joint disease?</i>	37
<i>Are the 5-HT_{2A} receptors on platelets of significance in joint disease?</i>	38
<i>Are the 5-HT_{2A} receptors on other cell types of significance in joint disease?</i>	39
<i>What is the effect on joints of 5-HT_{2A} antagonism and of decreased levels of 5-HT_{2A} receptors?</i>	40
<i>Are 5-HT_{2A} receptors of significance in joint disorders other than RA and joint ADRs?</i>	41
<i>Limitations and possible bias of the studies</i>	42
<i>Limitations regarding the cohorts used</i>	42
<i>Limitations regarding the genetic studies</i>	43

<i>Limitations with the epidemiologic study</i>	45
Concluding remarks	48
Conclusions	49
Acknowledgements	50
References	52

Abstract

Background

Serotonin (5-HT), an amino acid derivative and neurotransmitter, has for long been studied in relation to inflammation. It is an endogenous ligand for several different types of serotonin receptors. The serotonin receptor 5-HT_{2A} has been reported to have a role in the pathophysiology of arthritis in animal experiment models. However, no studies into this subject have been reported in man.

Objective

The objectives of this project were firstly, to examine possible associations for the 5-HT_{2A} receptor and also for the gene (HTR2A) encoding for the receptor with arthritis in man and secondly, to explore possible mechanisms underlying such associations.

Methods

The density and affinity of platelet 5-HT_{2A} receptors were determined in 43 patients with a common inflammatory joint disease, *i. e.*, rheumatoid arthritis (RA), in comparison with matched controls using a radio-ligand assay. The effects of treatment with prednisolone on 5-HT_{2A} density and affinity were also examined in 27 individuals diagnosed with polymyalgia rheumatica before and after start of treatment. In addition, possible candidate HTR2A genes were studied in relation to RA in two Swedish cohorts incorporating a total of 2450 RA patients. Furthermore, a register study using reports of joint symptoms as adverse drug reactions (ADRs) in the Swedish and the WHO ADR databases was undertaken. The proportion of reports concerning joint symptoms in relation to all ADR reports and to sales figures was analysed for 5-HT_{2A} blocking atypical antidepressant substances compared with another group of antidepressants, *i. e.*, selective serotonin re-uptake inhibitors (SSRIs), used for similar clinical indications.

Results

The mean density of 5-HT_{2A} receptors in RA patients was significantly lower than in controls, 45.3 versus 57.4 fmol/mg protein ($p = 0.004$). There was no significant difference in affinity. Variation of four single nucleotide polymorphisms (SNPs) (rs6314, rs1328674, rs6313 and rs6311) in the HTR2A gene was associated with RA, although not significantly so for all SNPs after testing for multiple comparisons. The proportion of joint symptoms reported as ADRs, relative to all ADRs was significantly higher for the 5-HT_{2A} blocking antidepressants compared with the SSRIs in both databases ($p < 0.001$). In the Swedish material the comparison of ADRs was also related to sales figures, showing a considerable higher frequency of joint symptoms for the 5-HT_{2A} antagonists ($p < 0.001$). The density of 5-HT_{2A} receptors increased after treatment with prednisolone in 23 out of 27 individuals. The mean density at baseline was 45.2 versus 64.9 fmol/mg protein at the end of the study ($p=0.001$). There were no significant differences in affinity during the treatment period, although a low affinity at baseline was a predictor for higher density following treatment with prednisolone.

Conclusions

The density of 5-HT_{2A} receptors, reflecting the number of receptors, was markedly reduced in a cohort of patients with RA from Northern Sweden. This may depend, at least in part, on an association between RA and certain HTR2A SNPs. Genetically determined or acquired low levels of accessible 5-HT_{2A} receptors may contribute to susceptibility for development of joint symptoms, not only in RA but more generally, *e. g.*, joint ADRs caused by 5-HT_{2A} blocking atypical antidepressants. The benefits of treatment with glucocorticoids may, at least partially, be mediated by an effect on 5-HT_{2A} receptors.

Abbreviations

5-HT	Serotonin
5-HT _{2A}	Serotonin receptor of type 2A
ACR	American College of Rheumatology
ACTH	Adrenocorticotrophic hormone
ADR	Adverse drug reaction
anti-CCP	Anti-cyclic citrullinated peptide
BCPNN	Bayesian Confidence Neural Network
B _{max}	Maximum binding, density
CNS	Central nervous system
COX	Cyclooxygenase
CRH	Corticotropin releasing hormone
DMARDS	Disease modifying anti rheumatic drugs
Dpm	Disintegrations per minute
ESR	Erythrocyte sedimentation rate
GCA	Giant cell arteritis
GPCRs	G protein coupled receptors
HLA	Human leukocyte antigen
HPA	Hypothalamic-pituitary-adrenal axis
HW	Hardy-Weinberg
K _d	Dissociation constant, affinity
LD	Linkage disequilibrium

LSD	Lysergic Acid Diethylamide
MI	Myocardial infarction
MPA	Medical product agency
PCR	Polymerase chain reaction
PET	Positron emission tomography
PMR	Polymyalgia rheumatica
RA	Rheumatoid arthritis
RCT	Randomised controlled trials
RF	Rheumatoid factor
RT-PCR	Reverse transcriptase-polymerase chain reaction
SCB	Statistiska centralbyrån, Statistics Sweden
SERT	Serotonin transporter
SSRI	Selective serotonin reuptake inhibitors
SWEDIS	Swedish Drug Information System
TCA	Tricyclic antidepressants
T-cells	T-lymphocytes
TNF	Tumor necrosis factor
TRC	Thrombocyte count
WHO	World Health Organization

List of studies

This thesis is based on the following studies, which will be referred to by the appropriate Roman numeral:

- I Kling A, Rantapaa-Dahlqvist S, Stenlund H, Mjorndal T. **Decreased density of serotonin 5-HT_{2A} receptors in rheumatoid arthritis.** Ann Rheum Dis. 2006;65(6):816-9.
- II Kling A, Seddighzadeh M, Arlestig L, Alfredsson L, Rantapaa-Dahlqvist S, Padyukov L. **Genetic variations in the serotonin 5-HT_{2A} receptor gene (HTR_{2A}) are associated with rheumatoid arthritis.** Ann Rheum Dis. 2008;67(8):1111-5.
- III Kling A, Danell-Boman M, Stenlund H, Dahlqvist R. **Association between the use of serotonin receptor 2A-blocking antidepressants and joint disorders.** Arthritis Rheum. 2009;61(10):1322-7.
- IV Kling A, Mjorndal T, Rantapaa-Dahlqvist S. **Glucocorticoid treatment increases density of serotonin 5-HT_{2A} receptors in humans.** Psychoneuroendocrinology. 2012. Epublished Nov 9

Populärvetenskaplig sammanfattning

5-HT_{2A} – en serotoninreceptor med möjlig betydelse för ledsjukdomar

Bakgrund till forskningsprojektet

Serotonin (5-HT) är en så kallad signalsubstans. När serotonin binder till så kallade serotoninreceptorer som finns på utsidan av flera av kroppens celler, bland annat nervceller och blodplättar, så uppkommer en signal som kan påverka olika fysiologiska skeenden i kroppen. En av serotoninreceptorerna har fått namnet 5-HT_{2A}. Serotonin och olika serotoninreceptorer har studerats i relation till psykiatriska sjukdomar, men också i relation till inflammation. Serotoninreceptor 5-HT_{2A} har från tidigare djurexperimentella studier rapporterats ha en roll i uppkomsten av ledinflammation. Dock har det inte studerats om 5-HT_{2A}-receptorn har betydelse för uppkomsten av ledsjukdom hos människor. Det har sedan tidigare visats att det finns flera genetiska variationer i HTR2A genen som kodar för serotoninreceptor 5-HT_{2A}. I vissa studier har dessa genetiska variationer visat association till olika sjukdomar (bl a psykiatriska sjukdomar, fibromyalgi).

Det är sedan länge känt att kortison gett som läkemedel minskar symtomen av ledinflammation.

Mål med forskningsprojektet

Målen med detta projekt har dels varit att undersöka om 5-HT_{2A}-receptorn och även genen som kodar för denna receptor (HTR2A) verkar ha betydelse för risken att få inflammatorisk ledsjukdom eller ledsymtom för människor. Dels undersöka om kortisonmedicinering påverkar antalet 5-HT_{2A}-receptorer.

Metoder som använts i detta forskningsprojektet:

De ovan beskrivna målsättningarna kom att undersökas med följande metoder:

Bindningsförmågan till 5-HT_{2A} receptorer på blodplättar för ett visst läkemedel uppmättes för patienter med den vanliga inflammatoriska ledsjukdomen reumatoid artrit och för kontrollpersoner utan denna sjukdom. Bindningsförmågan till 5-HT_{2A} receptorer på blodplättar uppmättes också för patienter med en annan reumatologisk sjukdom (polymyalgia rheumatica, som ibland kallas mjukdelsreumatism) före och efter behandling med kortisonpiller. Detta gjordes för att studera kortisonets effekter på 5-HT_{2A} receptorer.

Olika genetiska varianter som kodar för serotoninreceptorn 5-HT_{2A} analyserades också för patienter med reumatoid artrit och för kontrollpersoner.

Förekomsten av ledbiverkningar av antidepressiva läkemedel som påverkar 5-HT_{2A} receptorer analyserades med hjälp av det svenska registret över läkemedelsbiverkningar och WHO:s internationella register över läkemedelsbiverkningar

Resultat

Antalet 5-HT_{2A} receptorer var i genomsnitt mycket lägre hos 43 personer som hade reumatoid artrit än nivån av dessa receptorer hos kontrollpersoner. Åtminstone en del av detta skulle kunna förklaras av genetisk variation i genen som kodar för 5-HT_{2A}-receptorn. Andelen av vissa varianter i denna gen var nämligen vanligare hos patienter med reumatoid artrit än hos kontrollpersoner.

När biverkningsrapporter analyserades med avseende på andelen av ledbiverkningar relaterat till andra typer av biverkningar, visade det sig att ledbiverkningar var mer vanligt förekommande efter medicinering med antidepressiva som blockerar 5-HT_{2A} receptorer än för andra antidepressiva som inte gör det. Denna skillnad framkom också när ledbiverkningar från antidepressiva relaterades till försäljningssiffror för dessa läkemedel i Sverige.

Efter cirka en veckas behandling med kortisontabletter ökade antalet 5-HT_{2A} receptorer, och efter ungefärligen en månad var nivån av 5-HT_{2A} receptorer påtagligt högre än innan kortisonbehandlingen påbörjades.

Konklusion

I projektet har ett möjligt samband mellan nivån av antalet 5-HT_{2A} receptorer och reumatoid artrit påvisats.

Antidepressiva läkemedel som blockerar 5-HT_{2A} receptorer visade sig ha fler rapporter om ledbiverkningar jämfört med andra antidepressiva läkemedel.

I studien sågs också ett samband mellan variation i den gen som kodar för serotoninreceptor 5-HT_{2A} och reumatoid artrit.

En uppreglering av antalet 5-HT_{2A} receptorer sågs efter kortisonbehandling.

Introduction

The serotonin system

In the body there exists a lot of communication networks between different cells and tissues, which are sometimes called pathways. Often these pathways include receptors located on outer cell membranes. Ligands are substances that bind to receptors and some ligands are endogenous, *i. e.*, produced in and excreted in the body. There are many different types of receptors. One group of receptors has been named serotonin receptors, since they all have been assigned for the same endogenous ligand, namely serotonin. Receptor signalling and regulation of receptors could occur when ligands bind to the receptors. A ligand can either increase signalling (agonist), decrease signalling (inverse agonist) or block the accessibility to the receptor for other ligands (antagonist). Signalling in the serotonin system has sense for a lot of both central and peripheral functions, *e. g.*, mood, sleep, nociception, motor and attention control, appetite, cognition, thermoregulation, sexuality, haemostasis and regulation of the intestine [1].

Serotonin

Serotonin (5-hydroxytryptamine, 5-HT) was first discovered in enterochromaffin cells in the gut during the 1930s of Erspamer who called the substance enteramine. In 1948 a vasoconstrictor substance was discovered and named serotonin and was some years later shown to be identical to the enteramine [2-4].

Serotonin is an indole amine and a derivative from the essential amino acid tryptophan. Tryptophan hydroxylase is the rate-limiting enzyme in the synthesis. Rate-limiting is also the transport of tryptophan from the blood to the CNS, since several different amino acids compete for the same transport protein. Serotonin is metabolised by the enzyme monoaminooxidase (MAO) towards the main end product 5-hydroxyindole acetic acid (5-HIAA). A significantly smaller portion of the serotonin in the body, which is metabolized via other enzymes becomes melatonin, a substance which has importance for the regulation of the day and night rhythm [5].

Serotonin is abundant in enterocromaffin cells of the gastrointestinal tract, in granule in platelets, and as a neurotransmitter in the CNS [2, 6, 7]. Of the approximately total amount of 10 mg serotonin in the human body, 90%-95% has been estimated to be located in the gastrointestinal tract, while of the remaining part 8% is found in platelets and 1%-2% in CNS [8, 9]. There is a membrane protein, called serotonin transporter (SERT), that actively transports serotonin into cells [10].

Serotonin receptors

In 1950s it became clear that there was more than one type of serotonin receptor, which at that time were called D and M receptors [11]. The D receptors would later be called 5-HT₂ receptors [12]. Until now, several additional serotonin receptor types have been separated and identified by pharmacological analysis and cDNA cloning. There is now estimated to be 14-16 different subtypes of serotonin receptors which are divided into 7 main groups (5-HT₁-5-HT₇), of which at least major group 1, 2 and 5 have two or more subtypes included in the group. Subtypes are indicated by a letter after the main number, *e. g.*, 5-HT_{2A} [13-15]. All the serotonin receptors are G-protein coupled receptors, with the exception of the 5-HT₃ receptors which are ligand-gated ion channels [13, 14]. The different subtypes seem to have different expression and function, although there are overlaps [16, 17].

The serotonin system and platelets

Platelets are irregular cell fragments without a cell nucleus derived from their progenitors, the megakaryocytes. The average lifespan of platelets is reported to be 9-10 days [18]. Serotonin is not synthesised in platelets, although these cells are rich in serotonin content. This is because circulating serotonin from the blood, originating from the gastrointestinal tract is actively transported into platelets through SERT and stored into granule called dense bodies. The platelet SERTs therefore have a great importance for the regulation of serum levels of serotonin. When platelets become activated and start to aggregate, they release serotonin. Besides SERT platelets also possess 5-HT_{2A} receptors [19-21].

Serotonin receptor 5-HT_{2A}

The 5-HT₂-receptor has later been separated into 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors [15, 22-24]. The 5-HT_{2A} receptor is a G-protein coupled receptor (GPCRs) which is excitatory through activating phospholipase C (PLC) to generate diacylglycerol, a cofactor for activating protein kinase C (PKC) and to hydrolyse phosphatidylinositol bisphosphate to inositol trisphosphate. Inositol trisphosphate serves as a second messenger that increases intracellular calcium, thereby activating PKC, which in turn can affect other proteins thereby propagating biochemical signals. However, 5-HT_{2A} receptors could also activate phospholipase A₂ which promotes the release of arachidonic acid. These two pathways are considered to be the main pathways for 5-HT_{2A} receptor signal transduction [5, 25-27]. In addition, several other proteins and pathways could be influenced by 5-HT_{2A} receptor stimulation. Different ligands seem to affect different pathways. This functionally selective diversity leads to great complexity [26].

Beside the designated endogenous ligand serotonin, a lot of pharmaceutical substances that act as ligands for the 5-HT_{2A} receptor have been discovered or developed. Several of the ligands that are agonists are also hallucinogens, *e.g.*, LSD, while some of the antagonists commercial available have been used in treatment of depression and psychosis, *e.g.*, mianserin and clozapine, respectively [13].

Localisation/expression of 5-HT_{2A} receptors

The 5-HT_{2A} receptor is a cell surface membrane receptor [26], which has been detected at receptor protein level and/or mRNA level in many different tissue types both in humans and in animal models. 5-HT_{2A} receptors are highly expressed in many parts of the CNS, especially the cerebral cortex [17, 25, 28]. In central nerve tissues 5-HT_{2A} receptors have been found to be mainly postsynaptically localised, although presynaptic receptors have also been detected [29]. 5-HT_{2A} receptors have also been demonstrated to be localised on peripheral sensory axons of nerve cells from rats [30]. 5-HT_{2A} receptors have in addition to neuronal cells been shown to be present in many other tissues and cell types, *e. g.*, platelets [31], smooth muscle cells in blood vessels [32] and in lungs [33], different cell types in the bowel [34], cardiac myocytes [35], immune cells [36-38], cultured fibroblasts [39], ovary [40], skeletal muscle [41], eyes [42], kidney [43] and urinary tract [44].

Functions of the 5-HT_{2A} receptor

5-HT_{2A} receptors have various functions in connection to the different tissues/cells where they are located. In the CNS 5-HT_{2A} receptors play a role in neuronal regulation of social and cognitive functions [26]. In peripheral nerves 5-HT_{2A} receptors has been suggested to have a role in perception of pain [45]. Stimulation of platelet 5-HT_{2A} receptors with serotonin leads to a mild aggregating effect of the platelets which, however, is strengthened if the platelets concurrently are exposed to collagen. Furthermore, the stimulation contributes to the recruitment of further platelets to the aggregation process [21, 46, 47]. Serotonin binding to 5-HT_{2A} receptors of injury-exposed smooth muscle cells in blood vessels induces a vasoconstriction. This effect is enhanced by thromboxane A₂, kinins and vasoactive peptides. Both the effects on platelets and the effects on blood vessels contribute to haemostasis, respectively. Also in the smooth muscle cells in the gut, serotonin binding to 5-HT_{2A} receptors gives a stimulation resulting in a contracting effect [5].

Regulation of the 5-HT_{2A} receptor

The regulation of the 5-HT_{2A} receptor is not fully understood, although it seems obvious that changes in the numbers of receptors expressed, *i. e.*, the receptor density, is of major importance [27]. Multiple factors contribute to the complex regulation of the functional activity and the expressed number of 5-HT_{2A} receptors.

The 5-HT_{2A} receptor displays a constitutive activity, which means that there could be receptor signalling without ligand binding to the receptor. This constitutive activity could be decreased by inverse antagonists [48, 49]. The regulation becomes even more complex, since it, in addition, seems that different cellular signalling pathways could react in different ways following stimulation with the same ligand depending on the cell type [49].

Besides gene level, the 5-HT_{2A} receptor has been shown to be regulated on both transcriptional [50, 51] and receptor level [52]. The 5-HT_{2A} receptor can, like other GPCRs, be regulated in the short-term by agonist or antagonist stimulation, but can also be regulated in long-term following prolonged or repetitive stimulation. Chronic stimulation of several different agonists and antagonists at the 5-HT_{2A} receptor has in most studies resulted in a down-regulation of the receptor. Down-regulation by agonists is also a well-known mechanism for other GPCRs, although down-regulation by

antagonists is unlike other GPCRs and this phenomenon has been mentioned as paradoxical regulation [53]. Whether this regulation occurs at the mRNA level or is posttranscriptional, *i. e.*, receptor internalization, is not clear. This long-term regulation has been demonstrated for some tricyclic antidepressants, *e. g.*, amitriptyline and some neuroleptics, *e.g.*, clozapine [54]. The long-term downregulation is also described both *in vitro* and *in vivo* for the 5-HT_{2A} blocking atypical antidepressant mianserin [55-57], although in one *in vivo* study of platelets, mianserin caused an upregulation after a treatment period of five consecutive days in healthy volunteers [58]. It is unclear whether 5-HT-selective reuptake inhibition in general results in downregulation of 5-HT_{2A} receptors after chronic treatment, since the results are inconsistent [54].

Upregulation of 5-HT_{2A} receptors have been demonstrated as a result of stress in animals [59-63] and after glucocorticoid supply [64-70] in studies performed in rodents.

Connections between the 5-HT_{2A} receptor and the HPA-axis

The hypothalamic-pituitary-adrenal axis (HPA-axis) is a neuroendocrine system, which regulates many parts of the physiologic processes and responses in the body. The system is activated by stress and inflammation leading to secretion of endogenous corticosteroids. In humans, one of these corticosteroids is the active steroid hormone cortisol, acting on usually intracellular glucocorticoid receptors. In rats, corticosterone is the equivalent to the human cortisol.

Release of serotonin has been shown to increase the release of cortisol and adrenocorticotrophic hormone (ACTH). This has been demonstrated to take place at the hypothalamic level where synaptic connections between serotonergic nerve terminals and corticotropin releasing hormone (CRH) releasing neurons are present. In addition, there are studies indicating that a regulation of the HPA axis by serotonin may also exist in the anterior pituitary gland and in the adrenals. Serotonin 5-HT_{2A} receptors are located in the paraventricular hypothalamic nucleus, in the pituitary gland and probably also in the adrenals; and have been found to stimulate the HPA-axis on different levels [71-73].

While the serotonin system regulates the HPA-axis, corticosteroids have been found to regulate the serotonin system via serotonin synthesis and the various serotonin receptors [73, 74]. Administration of systemic exogenous

corticosteroids has in most studies resulted in an significant increase of 5-HT_{2A} receptors in rat models [64-70], although not in one study [75] (Table 1).

Table 1. Previous studies of the effects on 5-HT_{2A} receptors following glucocorticoid administration in rat models.

Author	Reference	Study design	Resultats on 5-HT _{2A}
Crayton 1996	[75]	Corticosterone 1mg/kg/day intraperitoneal for 7 days to 9 rats in treatment group and 10 rats in control group.	B _{max} ns K _d ns
Fernandes 1997	[64]	Corticosterone pellet 100mg SC for one week to 16 rats in treatment group and control group, respectively.	B _{max} ↑ sign K _d No information
Jitsuiki 2000	[65]	Dex 1mg/kg/day injected SC for 14 days to 10 rats in treatment group and 12 rats in control group.	B _{max} ↑ sign K _d ns
Katagiri 2001	[66]	Dex 1mg/kg/day injected SC for 14 d per treatment group and control group	B _{max} ↑ sign K _d ns
Kozuru 2000	[69]	Dex 1mg/kg/day injected SC for 14 days to 11-12 rats in treatment group and control group, respectively.	B _{max} ↑ sign K _d ns
Kuroda 1993	[68]	Dex 1, 2, 5 mg/kg/day, respectively, injected SC for 10 days to 8, 7, 5 rats in treatment group, respectively and 12 rats in control group.	B _{max} ↑ sign for all treatment groups K _d ns

Kuroda 1992	[67]	Corticosterone 20mg/kg and 50mg/kg, respectively injected SC for 10 days to 8 rats in treatment group, respectively, and 8 rats in control group.	B_{\max} ↑ sign for all treatment groups K_d ns
Takao 1997	[70]	Corticosterone 50mg/kg/day injected SC for 14 days to 8 rats in treatment group and control group, respectively.	B_{\max} ↑ sign K_d No information

Abbreviations: Dex, dexamethasone; SC, subcutaneous; ↑, significant increase; ns, not significant

HTR2A – The gene encoding for the serotonin receptor 5-HT_{2A}

The gene encoding for the 5-HT_{2A} receptor is called HTR2A. The rat HTR2A gene was cloned in 1988 [76] and the human HTR2A was cloned in 1991. The human HTR2A gene shares approximately 87% amino acid homology with the rat HTR2A gene [77]. An amino acid in position 242 has been shown to differ between man and rat, and furthermore this difference results in a substantial influence on the pharmacological properties for binding of several ligands for the 5-HT_{2A} receptor [78, 79].

The HTR2A gene has been localised to the human chromosome 13q14-q21 and consists of three exons and two introns [80, 81]. The HTR2A gene carries a large number of genetic variations of the type single-nucleotide polymorphism (SNP). Several of these SNPs have been studied in relation to illnesses, especially psychiatric diseases. The three most studied SNPs are rs6313 (T102C) and rs6314 (His452Tyr) in exon 1 and 3, respectively, and rs6311 (-1438G/A) close to the promoter region. About 50 additional SNPs have been studied, of which many are located in introns [82]. Some of these intronic SNPs may be of importance for splicing or enhancement of exogenic parts of the gene.

Rs6311 has shown to be in the HTR2A promoter region [83], and the variation may affect promoter activity under certain conditions [84]. However, it has also been hypothesised that this SNP could be in linkage disequilibrium (LD) with other functional polymorphisms in the promoter

region [85]. The variation in the rs6314 results in an amino acid substitution from histidine to tyrosine, leading to a decrease in the signalling function [86, 87]. A difference in receptor density, with higher density for the His/His variant, was also found in one study, although the difference was not significant [87]. Rs6313 is a so-called silent mutation or synonymous SNP, meaning that all three possible genotypes (CC, CT and TT) are resulting in the same amino acid, namely serine [88]. Nevertheless, it has been proposed that the genotypes give rise to different quantities of receptor protein, although receptor binding studies have been inconsistent in this case [89-91]. Furthermore, studies in rs6313 heterozygote (CT) individuals have revealed differences in the amount of 5-HT_{2A} receptor mRNA depending on which allele, “C” or “T”, that was transcribed. The “C” allele yielded a significantly lower expression of mRNA compared with mRNA originating from transcription of the “T” allele [92, 93].

Methods to analyse 5-HT_{2A} receptors

There have been different methodological approaches to examining 5-HT_{2A} receptor status as described below.

Radioligand methods

Radioligand methods are often based on estimations of two measures of 5-HT_{2A} receptor status. Firstly, receptor density (B_{\max}), which represents the total number of 5-HT_{2A} receptors available for binding to an appropriate ligand. Increased and decreased values of B_{\max} could be due to upregulation and downregulation of receptors, respectively. In sense of function this may mirror increased and decreased total capacity in signalling through 5-HT_{2A} receptors. Second, the dissociation constant (K_d), *i. e.*, the half maximal occupancy of the receptors, a measure of the strength or affinity of which the receptors bind a ligand. Changes in B_{\max} often leave K_d unaltered. In practical uses an appropriate radioligand is incubated with a homogenate of platelet cell membranes. Another ligand that binds stronger is added and competes with the first ligand of the receptor binding sites. Specific binding to the receptor is defined as the total binding of the first ligand subtracted by the binding of the first ligand in the presence of the second ligand. Commonly used ligands in binding studies of 5-HT_{2A} receptors have been, *e. g.*, LSD, ketanserin, spiperone, 2,5-dimethoxy-4-iodoamphetamine (DOI) [26].

The radioligand methods has in different studies been performed in autopsy material from CNS, in platelets and later also in living humans in real time using positron emission tomography (PET). Radioligand binding studies performed in platelets have been used for long as a model for neurons in general and for analysing 5-HT_{2A} receptor status in neurons [94-103]. The validity of the model has been questioned, for example, in a study where 5-HT_{2A} receptor density was measured with PET simultaneously in platelets and in CNS in normal healthy volunteers. However, that study was small and different ligands were used for platelets and CNS, respectively, and furthermore B_{\max} and K_d were not used but a ratio between these values [104].

Methods based on genetic analysis

With techniques converting mRNA to cDNA, using the reverse transcriptase polymerase chain reaction (PCR), HTR2A mRNA levels have been quantified and used as an indirect measure of expressed 5-HT_{2A} receptors. HTR2A mRNA has also been shown to correlate with 5-HT_{2A} receptor density [105].

In genetic association studies various SNPs of the HTR2A gene have been examined in relation to many, especially psychiatric, diseases.

Measurements of ion flows

Ion flows of Ca^{2+} or of inositol trisphosphate have been measured after stimulation of cells in vitro with different ligands. Calcium ion flows in response to stimulation with serotonin in platelets have been shown to correlate with 5-HT_{2A} receptor density [106].

Other methods

Western blot with an affinity-purified antibody and subsequent examination of 5-HT_{2A} receptor protein samples by electrophoresis has also been described [92] as well as immunohistochemical staining of 5-HT_{2A} receptors [107].

The 5-HT_{2A} receptor and psychiatric diseases

The 5-HT_{2A} receptor has been a subject for studies concerning psychiatric diseases. These studies have focused on three fields: Firstly, density of 5-HT_{2A} receptors has in some studies been shown to be affected in psychiatric diseases. Secondly, drugs affecting 5-HT_{2A} receptors are effective in the treatment of some patients with psychiatric diseases. Thirdly, polymorphisms in the HTR2A gene have in some studies been associated with psychiatric diseases. However, the mechanisms of how 5-HT_{2A} receptor status influences mental health remain to be clarified.

An increased density of 5-HT_{2A} receptors has been found using ligand binding analysis of cerebral tissue [108, 109] and of platelets [110-113] in studies of depression, and of cerebral tissue [114-117] and of platelets [112, 118] from individuals having attempted or committed suicide. However, there are also conflicting results showing no significant increases in 5-HT_{2A} receptor density in patients with depression [119] and in suicide attempters [120].

Genetic association studies between polymorphisms in the HTR2A gene and depression or suicidal behaviour seem in conclusion to be weak, if existing. The best support for any association seems to be for rs6311 and suicidal behaviour [121-123].

For patients with schizophrenia, ligand binding studies using platelets have also shown an increased density of 5-HT_{2A} receptors [124-127] while studies performed on cerebral tissue are somewhat contradictory [124]. The results of PET studies are also contradictory [128-131].

Genetic studies of possible associations between variations in the HTR2A gene and schizophrenia are numerous. An European meta-analysis supported an association between schizophrenia and rs6313 [132]. In another meta-analysis this association was weak and more pronounced in the European studies included [133]. However, a later Chinese meta-analysis found no overall association for rs6313, although there was a small significant association to the C allele when the European cohorts were considered separately [123].

Studies of possible effects on 5-HT_{2A} receptor status induced by antidepressants or neuroleptics in patients receiving these drugs have given conflicting results and an inconclusive picture [54, 124].

Additionally, there are studies indicating that 5-HT_{2A} receptors may have sense for other psychiatric diseases, *e. g.*, eating disorders, anxiety disorders, attention deficit hyperactivity disorder (ADHD) [121, 122].

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a systemic inflammatory joint disease with a prevalence of 0.5% to 1% among countries with a predominant Caucasian population as in Sweden [134]. The disease is characterised by progressive synovitis, which often ends up in a symmetric polyarthritis of hands and feet with destruction of joints resulting in impaired function. From a immunohistopathologic view extravasations of joints with infiltration of inflammatory T-cells, B-cells and macrophages could be seen. Activated T-lymphocytes in turn activate macrophages which synthesise and secrete pro-inflammatory cytokines, such as TNF alpha and interleukin-1 which among other biological substances increase the endothelial permeability leading to more infiltration of inflammatory cells to the joint. In addition to synovitis extraarticular manifestations may occur, *e. g.*, anemia vasculitis, rheumatic noduli, keratoconjunctivitis sicca, mononeuritis, pleuritis, pericarditis and enlarged lymph nodes [135].

RA has been defined according to established classification criteria, *i. e.*, the 1987 American College of Rheumatology (ACR) criteria [136] and the 2010 ACR/EULAR RA Classification Criteria [137]. RA is a so-called complex disease which means that many different genetic, hormonal and environmental factors are supposed to increase the risk of developing the disease. A number of gene loci that increase the susceptibility for RA have been identified so far [138]. The strongest association of genetic factors has been shown for the HLA-DRB1 shared epitope (HLA-SE). Cigarette smoking is an environmental factor that has been shown to increase the risk for developing RA and, in addition, contribute to a strong gene environmental interaction with HLA-SE [139].

Common laboratory markers of RA are in addition to markers of systemic inflammation, such as increased erythrocyte sedimentation rate (ESR), increased C-reactive protein (CRP), thrombocytosis and anaemia, also certain auto antibodies, *i. e.*, rheumatoid factor (RF) and anti-cyclic citrullinated peptide antibodies (anti-CCP). Tests for RF and anti-CCP have a sensitivity of 60%-70%, whilst the specificity is higher. Presence of anti-CCP identifies a more severe and progressive disease [135].

The pharmacological treatment in RA is based on national and international guidelines [140, 141]. Classical disease modifying anti rheumatic drugs (DMARDs), *e. g.*, methotrexate, sulfasalazine, leflunomide, chloroquine, and biological agents, *e. g.*, TNF alpha inhibitors are used. Glucocorticoids, both in systemic and local preparations, have also for long been used in the treatment of RA. Systemic glucocorticoids seem to have good acute effects in reducing the inflammation and synovitis in many patients, and may also reduce joint destruction. However, the use has been restricted by the risks of adverse effects, *i. e.*, primarily osteoporosis, peptic ulcers, cataract and infections [142, 143]. COX-inhibitors are commonly used to palliate pain and could in cases of very mild RA be the only drug used for long periods of inactive disease. There are established scales for measuring disease activity and evaluating treatment effects, *e. g.*, Disease activity score (DAS) [144, 145].

Rheumatoid arthritis and schizophrenia

An inverse association between schizophrenia and RA has since long as well as recently been reported in epidemiologic studies [146-150]. Several explanations for this have been suggested, for example, that schizophrenic patients due to institutionalisation have fewer fractures, which could possibly affect the susceptibility to RA; or be due to underreporting of medical illness in schizophrenia [151, 152]. It has also been suggested that differences in HLA genotypes may account for the inverse association between the two diseases [153]. Differences in the serotonin system have also been suggested to have an impact on the inverse association between RA and schizophrenia [154].

Serotonin, 5HT_{2A} receptors and inflammatory joint disease

Serotonin has long been regarded as a mediator of inflammation [155, 156] and has in different models demonstrated a pro-inflammatory effect. Injection of serotonin into the hind paw of rats caused an acute dose-dependent oedema. When p-chlorophenylalanine (PCPA), a reversible inhibitor of tryptophan hydroxylase was injected intra-peritoneal at the debut of adjuvant-induced arthritis in rats, the inflammation was significantly reduced [157]. The 5-HT_{2A} receptor has been shown to be a mediator of serotonin-induced pain and inflammation in rats [158]. In animal models using rats, the magnitude of arthritis has also been shown to be limited by

blockade of 5-HT_{2A} receptors with ketanserin [159] and ritanserin [160]. Two other studies found that the SSRIs citalopram, fluoxetine and sertraline had antiinflammatory effects in rodent models of RA [161, 162].

Previous studies have shown lower levels of serotonin in platelets from patients with RA [163, 164]. There are studies that indicate that the release of serotonin from platelets is, at least in some cases, greater in patients with active or more severe RA disease [165, 166]. Another finding was that serum serotonin levels were higher in patients with rheumatoid factor positive RA [167]. Additionally, an old Italian study found that some RA patients had benefits from intra-articular injections with the partial 5-HT_{2A} agonist methysergide, although the effects on the disease were transitory [168]. On the whole, it is otherwise sparsely with published human studies in this field.

Polymyalgia rheumatica and Giant cell arteritis

Polymyalgia rheumatica (PMR) is a disease that manifests with stiffness, pain and tenderness in the proximal limb and/or girdle muscles. Giant cell arteritis (GCA), with symptoms such as headache, pulsating painful arteria temporalis and eye symptoms, is a vasculitis that affects large and medium-sized blood vessels, often arteria temporalis. The two diseases is overlapping and approximately 40% of patients with GCA have polymyalgia rheumatica, and about 10% with originally isolated PMR will after histologic examination be diagnosed with GCA. The etiology is not clear although an association to certain HLA-genotypes and environmental factors like infections have been suggested to play a role. In both diseases general symptoms such as tiredness, fever and weight loss are often seen. Onset is relatively fast and fully developed symptoms are seen after a few days to a few weeks. The disease begins very rarely <50 years and the highest incidence is seen around 80 years. ESR is often markedly elevated in both diseases. GCA has long had clearly defined diagnostic criteria identified by a biopsy, while PMR often becomes an exclusion diagnosis. Treatment with glucocorticoids is the first choice of treatment and gives in most cases a rapid alleviation of symptoms, which further strengthens the diagnosis. Start-up doses for patients with PMR is 15-20 mg prednisolone and with GCA 40-60 mg. Glucocorticoid dose is then quickly reduced for a few weeks, but a small maintenance dose of 2.5-7.5 mg may be necessary to remain remission for a long time [169, 170].

Adverse drug reactions

An adverse drug reaction (ADR) is an unwanted event caused or partly caused by a medical drug or with other words “an appreciably harmful or unpleasant reaction, resulting from an intervention related to the use of a medicinal product, which predicts hazard from future administration and warrants prevention or specific treatment, or alteration of the dosage regimen, or withdrawal of the product” [171]. The occurrence of all various ADRs must in general be considered as rather high, although it is often difficult to establish exact frequencies, because it is difficult to judge if the reaction is a consequence of drug treatment or only in coincidence with the treatment. Controlled trials are in general a good way to obtain more reliable figures of ADR frequencies. However, these trials are often very expensive and therefore mostly performed on newer drugs. In addition, they tend to focus on expected and serious ADRs. After the thalidomide tragedy in early 1960s the concept of pharmacovigilance took off. Since then, the spontaneous reporting of ADRs has been more encouraged, and is considered as important since not all, especially not rare, ADRs have been detected in clinical trials. National and international ADR databases have been built up and further developed, *e. g.*, the World Health Organization's (WHO) Adverse Reactions Database and the Swedish national database (SWEDIS, Swedish Drug Information System). Different methods for using spontaneous reported ADRs to find signals of possible ADRs have been performed.

Antidepressant drugs

This paragraph on antidepressant drugs is a brief summary of drugs being or having been in common use in Sweden. Monoamino oxidase inhibitors, which inhibit the metabolism of the monoamines noradrenaline and serotonin, were developed for the treatment of tuberculosis, but were also found to have an antidepressant effect. Tricyclic antidepressants (TCA), *e. g.*, amitriptyline, imipramine, and clomipramine, are inhibitors of noradrenaline and serotonin transport proteins, and additionally affecting several different monoaminergic receptors. The relative ratio between inhibition of noradrenaline and serotonin reuptake varies among different tricyclic substances. Later, newer antidepressants have been developed that lack TCA's effects on various receptors, *e. g.*, venlafaxine and duloxetine, or are more selectively noradrenaline reuptake inhibitors, *e. g.*, reboxetine and atomoxetine. The use of TCA has declined during the two past decades due to known serious adverse effects, especially cardiovascular effects. The

decline of the TCAs was in favour for the use of selective serotonin reuptake inhibitors (SSRI) which more selectively inhibit the serotonin reuptake and have less serious adverse effects. The first SSRI substance zimeldine was an exception in regard to this and was withdrawn shortly after its introduction due to a serious hypersensitivity syndrome. However, several other SSRI substances including fluvoxamine, paroxetine, citalopram, sertraline, fluoxetine and escitalopram were introduced later and have long time been the most commonly used drugs in the treatment of depression. In addition, some antidepressant substances acting as 5-HT_{2A} antagonists, *e. g.*, mianserin, mirtazapine and nefazodone, have been shown to exhibit antidepressive effects [172]. Nefazodone was withdrawn from the market due to adverse liver effects [173].

Aims

The overall aim of this thesis was to investigate whether serotonin 5-HT_{2A} receptors are of significance in joint disease. In order to examine this, there were a number of intermediate aims:

- To examine 5-HT_{2A} receptor density and affinity in patients with one common arthritic disease, *i. e.*, rheumatoid arthritis.
- To analyse genetic variation in the 5-HT_{2A} receptor gene in relation to one common arthritic disease, *i. e.*, rheumatoid arthritis.
- To investigate the occurrence of adverse drug reactions in joints following the use of 5-HT_{2A} receptor antagonists.
- To examine whether treatment with systemic glucocorticoids alters 5-HT_{2A} receptor density and affinity.

Subjects and Methods

Subjects

Patient group and control group in study I

In study I all available patients who fulfilled the 1987 ACR criteria for RA [136], and did not meet any of the exclusion criteria, and accepted participation in the study were consecutively recruited with the aid of reception lists at the Department of Rheumatology, University Hospital, Umeå. The number of excluded patients was high since many individuals had been treated with corticosteroids or were premenopausal women. In most cases the exclusion could be made based on information found in the patient's medical records. In addition, to ensure that no exclusion criterion was fulfilled both patients and controls had to meet a physician when they attended the research reception of clinical pharmacology in connection with blood specimen collection. An age- and sex-matched control group was recruited with the help of Statistics Sweden, which sent a letter with an invitation to participate as controls in the study. For each participating RA patient a letter was sent to 8-12 eligible control persons. The letter contained an invitation to participate in the study and information about the study and about the exclusion criteria. To minimize the risk that intraindividual variations in 5-HT_{2A} receptor binding attributed to seasonal variation would bias the final result, the blood sampling of all patients and controls was drawn close in time.

Patient group and control group in study II

Patients in the first discovery phase of the extended genetic study were a Northern Swedish study population of 292 patients with RA (207 women and 85 men) giving a female/male ratio of 2.44. Control samples in the first discovery stage of the extended study were a fusion of three different control materials and consisted of a total of 524 samples from 371 women and 153 men giving a female/male ratio of 2.42.

The cohort of patients and corresponding controls in the validation stage of the extended genetic study has been described previously [174, 175]. In short, patients with RA 18-70 years of age from the middle and southern parts of Sweden with a recent debut of RA fulfilling the 1987 ACR criteria for RA and diagnosed by a rheumatologist had been included in the study between May 1996 and February 2001. From this cohort called the Epidemiological Investigation of Rheumatoid Arthritis (EIRA) 1528 women and 630 men were included in the study, which gave a female/male ratio of 2.43. The control group consisted of 1068 samples from persons included in the EIRA study material. It was 798 women and 270 men, giving a female/male ratio of 2.96. The controls were randomly selected from the Swedish national population registry, taking into consideration the patient's age, sex and residential area.

PMR cohort used for the study of effects on 5-HT_{2A} receptors caused by medication with glucocorticoids in study IV

The subjects in Paper IV were patients with a newly diagnosed PMR or giant cell arteritis, or a combination of both diseases, or with a relapse in either of these diseases, who were not treated with systemic exogenous glucocorticoids during the previous three months. All available patients with any of these two diseases who did not meet the exclusion criteria and attended the Department of Rheumatology, University Hospital, Umeå were consecutively recruited to the study. The study was pre-planned to include at least 40 patients, although finally only 27 patients were included. Since there was no previous comparable human study, power calculations were difficult to perform. The number of 40 patients was based on the observation that studies on a limited number of subjects (>40) could give results that are hard to interpret due to, at least in part, intra- and interindividual variability [176]. However, the effects of interindividual variability became smaller in this study since each study participant was its own control. The reason that the number of subjects included in the study was less than the pre-planned number was due to matters of health care administrative nature. After the beginning of the study, patients with a PMR diagnosis were primarily referred to their district health care centre.

Ethical approvals

All human studies were approved by the Regional Research Ethics Committee, University of Umeå, Sweden (DNR 00-245, 00-403, 01-296, 02-174, 02-172). All the subjects included gave their informed consent.

Methods

[³H]LSD ligand binding of platelets in study I and IV

[³H]LSD binding in platelets was used to determine B_{\max} and K_d in study I and IV. The method has been used before and has been previously described [8, 96, 177] and is described in more detail in Paper IV. Some additional information and remarks not mentioned elsewhere should nevertheless, be given below.

Choice of ligands

Triated Lysergic Acid Diethylamide ([³H]LSD, N-methyl-³H) as a radioligand with spiperidole (spiperone) as the displacer to measure density and affinity of 5-HT_{2A} receptors, has for long been used [96]. The choice to use LSD as radio-ligand in this project was, besides the practical reason that the method already was optimised with this ligand at the laboratory, based on previous experiences of LSD as a more reliable ligand for 5-HT_{2A}-receptor studies in human platelets than ketanserin [8, 177]. [³H]LSD has been shown to bind 5-HT_{1A}- and 5-HT_{2A}-receptors to a similar extent [12] and one study reported that ¹²⁵I-labelled LSD was more specific to 5-HT_{2A}-receptors in relation to 5-HT_{1A} receptor sites than [³H]LSD [178]. LSD is also a ligand for 5-HT_{2C} receptors [179].

Spiperone have been reported to be able to discriminate between 5-HT_{2A} and 5-HT_{2C} receptors, with an affinity for 5-HT_{2A} receptors that was 38 times higher than for 5-HT_{2C} receptors. On the other hand, it has a high affinity for the 5-HT_{1A} receptors (Knight et al., 2004).

However, neither 5-HT_{2C} receptors nor 5-HT_{1A} receptors have been reported to be found on platelets. It seems that the 5-HT_{2A} receptors are

currently the only serotonin receptors which have been demonstrated to exist on platelets. Later further specific ligands have been explored [180], but these ligands were never considered for use in this project since the project at that time already had started.

Additional description concerning the [³H]LSD binding experiments

Incubation of the samples with [³H]LSD was performed in 96-well micro-plates with flat bottoms. Seven different [³H]LSD concentrations were used, *i. e.*, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0 and 2.5 nM. Each LSD concentration was measured in triplicate to give more reliable results. All triplicates were incubated with [³H]LSD alone and [³H]LSD in the presence of spiperone, respectively. One 96-well micro-plates contained two samples, including negative controls.

It is possible to run a maximum of four 96-well plates per working day, which means that a maximum of eight samples could be run per working day. In addition, only eight samples were possible to run during the centrifugation at 30 000 G during the preparation of the membrane protein solution. In order to minimise the possible variation due to the practical handling of the binding experiments, all three samples drawn from the same patient in study IV were analysed on the same working day, and similarly, patients were analysed along with controls of corresponding birth years in study I.

Rules for calculations of B_{max} and K_d for [³H]LSD ligand binding

Each LSD concentration with, as well as, without spiperone was analysed in triplicate and therefore three values of disintegration per minute (dpm) were obtained. Means were calculated for all triplicates. The mean of a triplicate of a given LSD concentration subtracted by the mean of the corresponding triplicate of LSD with added spiperone represents a value of specifically bound LSD to the 5-HT_{2A} receptors.

Since there were no previously established criteria for how to manage the calculation of the raw data from the binding experiments, the rules below were developed in connection to the project.

The mean values of the triplicates should increase for each concentration of LSD. No individual value in a triplicate was therefore permitted to be greater than the mean of the next triplicate or smaller than the mean of the previous triplicate. If this happened the individual value was not included in the calculation. If the coefficient of variance (CV%) for a triplet was above 15% and a single value differed markedly from the two other values in the triplicate, the deviant individual value was excluded from calculation. If the mean of a triplicate was less than previous triplicate or if two of the three individual values were less than the mean of the previous triplicate, or exceed the mean of the following triplicate, that deviating triplicate was completely removed from the calculation.

[³H]LSD ligand binding of lymphocytes

Since T-lymphocytes are considered to have an important role in RA, experiments to determine 5-HT_{2A} receptor density and affinity in the T-lymphocyte cell line Jurkat were also performed. The protocol for the membrane preparation and [³H]LSD ligand binding method of platelets were slightly modified. However, it was not possible to detect any specific saturable binding with the method used.

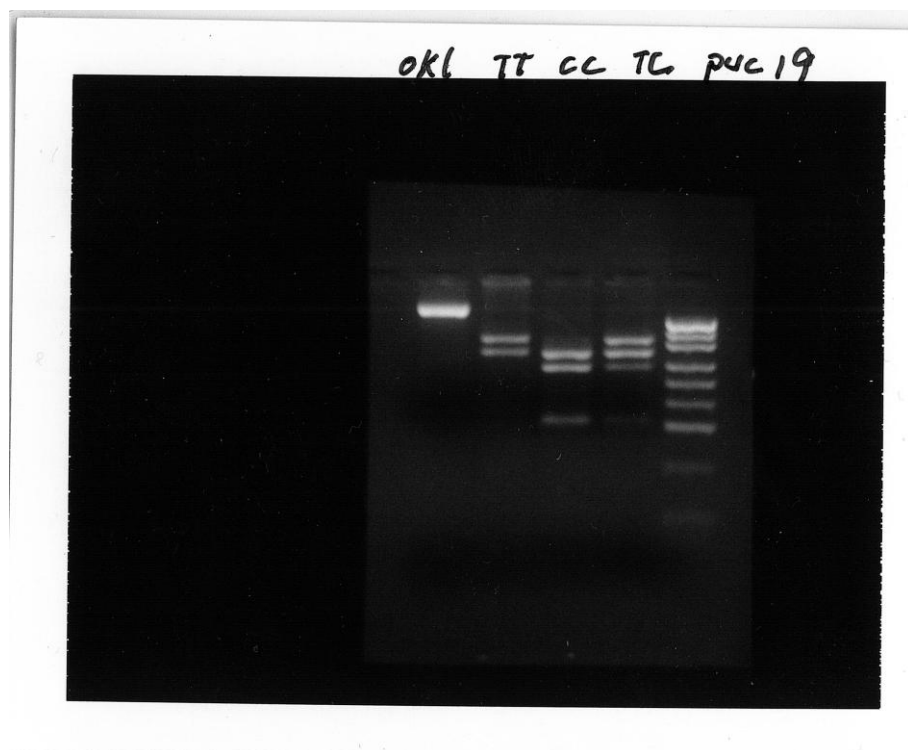
Genotyping in study I and II

In study I and in the discovery phase of study II the HTR2A SNP rs6313 was analysed with PCR in relation to RA. To avoid misjudgement of the analysis result in samples where the cleavage may have failed after digestion with the MSP I enzyme, primers were constructed in a way that permitted discrimination with two characteristic visible bands for each allele (figure 1). Detection of amplified gene products was first performed with silver staining in a polyacrylamide gel, which is more sensitive than ethidium bromide staining. These two methods, were compared before ethidium bromide staining in an agarose gel was used as standard method for staining of PCR gene products. About 10 samples were analysed with PCR in two different laboratories yielded the same result.

In the validation phase of study II all the three SNPs mentioned above, and commonly used in studies of psychiatric diseases (*i. e.*, rs6313, rs6314, and rs6311), and in addition five intronic SNPs (rs7997012, rs1328674, rs977003, rs2070037, rs2070040) were chosen to be analysed using TaqMan

technique. The method was performed at the Karolinska Institutet in Stockholm. To minimise laboratory errors and technical bias 163 samples were genotyped with both PCR and Taqman techniques with a 99% match between methods.

Figure 1. Ethidium bromide staining of amplified rs6313 products in an agarose gel.



Other experimental methods used

Experiments with flow cytometry were performed with the intention to examine 5-HT_{2A} receptor status in different types of blood cell. Due to some unspecific binding of the antibody the results were totally inconclusive.

Epidemiological method in study III

In study III the amount of ADRs in the joints possibly caused by 5-HT_{2A} blocking antidepressants was primarily compared to the amount of joint symptoms possibly caused by SSRI, and secondly also compared to the group of bisphosphonates. The choice of the SSRIs as the primary control group was based on the assumption that it was of importance that the individual drugs in the control group were prescribed on similar clinical indications and had been approved for clinical use in Sweden during approximately the same period of time. The latter is important since there may be differences in the awareness of ADRs and the tendency to report ADRs between different periods of time. The choice to use bisphosphonates as an additional control group was based on the existence of controlled clinical studies of bisphosphonates with a significant higher frequency of joint ADRs in the treatment group, and because it was difficult to find controlled clinical studies with a significant higher frequency of joint ADRs for any other group of drug.

As the start year of the study, 1990 was chosen since this included the approval dates of almost all the antidepressants of interest for the study. An exception was the SSRI substance zimeldine which was approved 1982, but withdrawn from the Swedish market 1983. In fact, there were many reports of joint disorders of zimeldine in the 1980s. However it turned out that this was related to a special syndrome called the zimeldine-induced hypersensitivity syndrome comprising not only arthralgia but also fever, myalgia and liver function disturbances and in some cases neurological disorders [181-183]. For practical reasons, 2006 was chosen as the end year of the study. At the time when the study was completed in early 2008, the figures of all ADRs for 2007 were not accessible in the databases.

There were more antidepressant substances than mianserin, mirtazapine and nefazodone that also exhibit a high affinity for the 5-HT_{2A} receptor. Firstly, there are other atypical 5-HT_{2A} blocking antidepressant substances, such as trazodone, which was not included since it has not been approved in Sweden. Secondly, the tricyclic antidepressant amitriptyline has a rather high affinity for 5-HT_{2A} receptors. However, amitriptyline was approved in Sweden as early as 1963, and since the propensity to report ADRs may have varied between different periods of time, it was decided not to be included in the study.

During the study period, a statement concerning reporting of ADRs, made by the Swedish central authority for medical drug issues the Medical Product Agency (MPA), was valid, and had the following sense: For all medications,

should all serious ADRs be reported, which included death, life-threatening reactions, permanent injury, long-term disabilities, hospitalisation, prolongation of existing hospitalisation, new unexpected reactions, drug interactions, and reactions which appear to be increasing in frequency or severity. In addition, during the first two years after the approval of a new drug, all suspected ADRs except those listed as common in the FASS-text (Farmaceutiska Specialiteter i Sverige, *i. e.*, Pharmaceutical Specialties in Sweden) must be reported.

The DDDs for the antidepressants, both 5-HT_{2A} antagonists and SSRI, are based on the assumed average dose for the treatment of the main indication in adults, *i. e.*, moderately severe depression [184]. The DDDs may differ from the average dose used in clinical practice, and may differ from the average dose used for other indications. The current DDDs are shown in table 2.

In the WHO database and in SWEDIS, ADRs are classified by specific reaction terms which are systemised into organ systems. From the main ADR reaction term “joint disorders” the specific reaction terms “arthralgia” and “arthritis” were chosen to be included in the study and in addition some quantitative smaller terms that was judged to be related to arthritis or arthralgia was identified and included. These terms in SWEDIS were “joint swelling” and “arthropathy”, and in the WHO database “arthritis aggravated”, “arthropathy”, and “synovitis”. In these databases some specific reaction terms are somewhat overlapping.

The Swedish database was checked for double reporting and in the few cases where this was found, the duplicates were excluded. However, as in other studies using the WHO database, there was no access to the identity of patients described in the reports, since those are stored in the different countries where the reports originated from, and consequently this database was not checked for possible double reporting. If an ADR report had more than one ADR term, *e. g.*, arthralgia and arthritis, which was the case in seven of the assessed reports, only the most serious term, was used in the statistical calculations.

Table 2. DDDs for the antidepressants included in study III.

Drug	DDD (mg) [184]
escitalopram	10
sertraline	50*
fluvoxamine	100**
fluoxetine	20
paroxetine	20
citalopram	20
nefazodone	400
mirtazapine	30
mianserin	60

*DDD before 1996 was 75mg. **DDD before 1996 was 150mg.

Statistical analyses

Paper I

In study I B_{\max} and K_d values were compared using two sample independent t-test and for comparing genotype and allele frequencies the χ^2 test were used. The control group was in agreement with the Hardy-Weinberg (HW) equilibrium ($p > 0.05$), while the RA study population differed slightly from this ($p = 0.047$). Pearson correlation coefficient was used to test for associations of B_{\max} with duration of disease, age at onset of RA, ESR and accumulated disease activity score, respectively.

Paper II

Genotype and allele frequencies between patients and controls were compared using the Chi-square or Fisher exact tests. Bonferroni correction was used to test for mass significance. The RA study populations in the discovery phase and the validation phase as well as all three control groups in the discovery study analysed separately and the control group in the validation study were in HW equilibrium.

Paper III

The statistical analyses for comparisons of ratios between joint ADR reports and the number of total ADR reports were performed with Chi-square test. The ratio between the number of joint ADR reports and the sales figures in DDD was calculated with a Poisson regression model.

Paper IV

Values for B_{\max} and K_d at baseline before start of treatment with prednisolone compared with B_{\max} and K_d values at the follow-up visits, were tested as related-samples using the Wilcoxon signed ranks test. Regression analyses were performed to analyse possible relations between K_d at start and changes in B_{\max} following treatment, as well as between changes in B_{\max} and accumulated prednisolone dose. Spearman's rho test was used to test for associations between changes in B_{\max} and possible confounders.

General

P-values <0.05 were considered as significant. Odds ratios (OR) or incidence rate ratios (IRR), with 95% confidence interval (95% CI) were calculated when appropriate. The SPSS software for Windows (SPSS; Chicago, IL), version 12.0 (study I-III) and 18.0 (study IV) was used for the statistical analyses. In addition, StatView was used in study II and III, HaploView 4.0 in study II, and Statcalc (EpiInfo, version 6) in study III.

Results

5-HT_{2A} receptor density and affinity (study I and IV)

5-HT_{2A} receptor density and affinity in RA (study I)

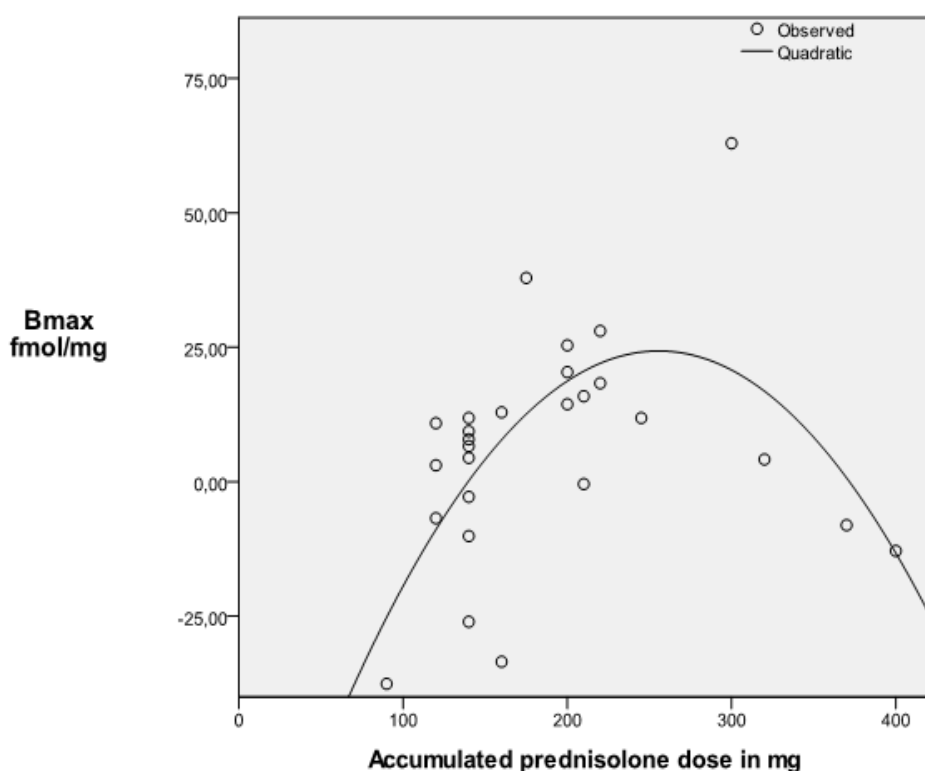
The 5-HT_{2A} receptor density was found to be lower in 43 patients with RA compared with a group of 49 sex- and age-matched controls. Means of B_{\max} were 45.3 vs 57.4 fmol/mg protein in the RA patients and controls, respectively, $p=0.004$. There was no significant difference between the two groups with respect to means of K_d 0.83 vs 0.79 nmol/l ($p=0.7$). The difference in mean B_{\max} was more pronounced among women and when the two groups were compared stratified for sex there was still a significant difference between the means of B_{\max} for women, $p=0.006$, but not between the means of B_{\max} for men, $p=0.25$. However, there was no significant difference in B_{\max} between women and men with RA ($p=0.5$). There was no relationship between B_{\max} and type of drug treatment, disease duration or age at onset of disease in the RA group. Both accumulated disease activity score and ESR on the day of sampling correlated inversely with B_{\max} within the RA group. Since ESR was also drawn from the controls the covariate could be tested in a regression model showing a negligible influence of ESR on the association between RA and B_{\max} .

5-HT_{2A} receptor density and affinity following glucocorticoid treatment (study IV)

In PMR patients starting a peroral glucocorticoid treatment with prednisolone, the mean B_{\max} of [³H]LSD binding to 5-HT_{2A} receptors at baseline before the start of treatment was 45.2 fmol/mg protein, and differed significantly ($p=0.001$) from 64.9 fmol/mg protein which was the mean B_{\max} at the second follow-up visit about one month later. This difference was also significant when men and women were compared separately, although there were no significant differences when men and women were compared with each other. None of the parameters age, sex, time to follow-up visits, ESR and TRC values or the emergence of psychiatric symptoms correlated with changes in mean B_{\max} . The difference between mean B_{\max} at baseline and at

the first follow-up visit, in mean about nine days later, was not significant. Accumulated prednisolone doses were significantly associated with changes in B_{\max} values from baseline to the first follow-up visit in a quadratic regression model ($p=0.003$), although not with changes in B_{\max} values at the second follow-up visit (Figure 2).

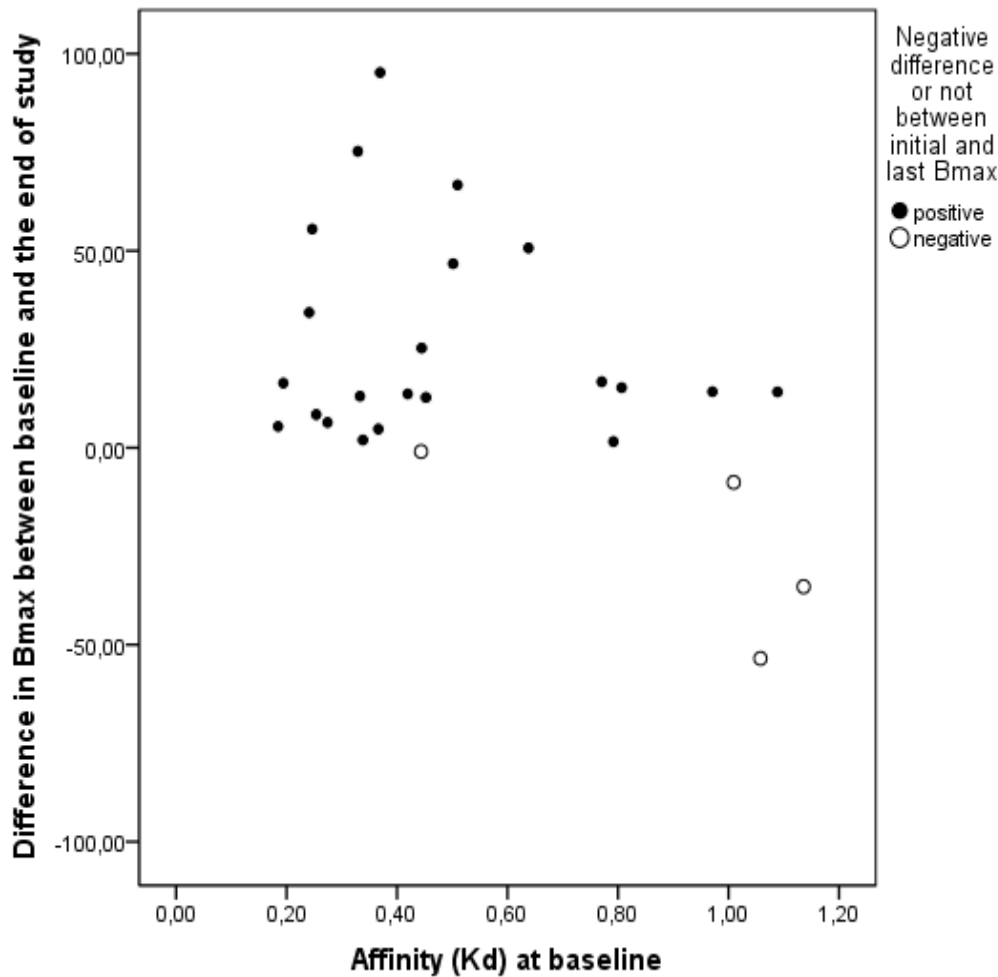
Figure 2. Changes in B_{\max} of 5-HT_{2A} receptors between baseline and first follow-up visit in relation to accumulated prednisolone dose.



There were no significant differences in mean K_d values between the different time points. However, the K_d value at baseline before starting of treatment was a significant predictor for the magnitude of the change in B_{\max} at the first ($p=0.048$) and at the second follow-up visit ($p=0.019$). Lower K_d

values were associated with a greater positive difference between the B_{\max} value at baseline and at follow-up visits (Figure 3).

Figure 3. 5-HT_{2A} receptor affinity (K_d) at baseline before start of treatment with prednisolone in relation to the difference between B_{\max} at baseline and B_{\max} at the end of the study.



Genetic HTR2A association studies (study I and II)

Already in study I a small but significant difference in rs6313 genotype distribution was detected. None of the patients with RA had the TT genotype, while six of the controls carried this genotype. The study was very small as a genetic study, but was encouraging in performing a larger study to explore this subject. In study II, comparisons of rs6313 genotype distribution between patients and controls were performed and replicated in another cohort. In both cohorts there were a significant difference between RA patients and controls in rs6313 genotype distribution (table 3), as well as in allele frequencies. The TT genotype was more uncommon among the RA patients. Three further SNPs (rs6311, rs1328674 and rs6314) were also found to be associated with RA, although only for one SNP, *i.e.*, rs1328674, did the significance for differences in allele frequencies remain after test for multiple comparisons. In addition, two haplotypes constructed from the four SNPs mentioned above showed a significant association with RA.

Table 3 Genotype distribution of rs6313 in two different Swedish cohorts of RA patients.

Groups	Genotypes n (%)			Total	p-value
Cohort from Northern Sweden	CC	CT	TT		
RA patients	148 (50.7%)	127 (43.5%)	17 (5.8%)	292	0.006 (0.002*)
Controls	244 (46.6%)	212 (40.5%)	68 (13.0%)	524	
EIRA cohort					
RA patients	883 (42.1%)	963 (46.0%)	249 (11.9%)	2095	0.011 (0.003*)
Controls	396 (38.5%)	473 (46.0%)	159 (15.5%)	1028	

*The p-values when only the frequencies of CC and TT genotypes were compared.

Joint symptoms as adverse drug reaction of 5-HT_{2A} antagonists (study III)

Joint ADRs in relation to all ADRs

In the Swedish database ADR reports of joint disorders represented 6.6 % of all ADRs reported for the 5-HT_{2A}-blocking atypical antidepressants. The corresponding figures for the SSRIs and bisphosphonates were 0.5% and 8.6%, respectively. Joint disorders were significantly more common ADR terms for the 5-HT_{2A} antagonists when compared with the SSRIs ($p<0.001$), while being on the same level as for the bisphosphonates ($p=0.22$). A comparison among the 5-HT_{2A} antagonists showed that mianserin had a significantly higher proportion of joint related ADRs than mirtazapine ($p<0.001$) but not when compared to nefazodone ($p<0.22$). The suspected joint ADRs disappeared upon discontinuation in most cases for all three groups of drugs and there was no significant difference in regard to that. It should be noted that the ADR reports concerning the bisphosphonates and joints included significantly more women and significantly older patients compared with the ADR reports of the 5-HT_{2A} antagonists. The median of appearance of joint ADRs was 21 days for the 5-HT_{2A} antagonists, and 50 % of the ADRs occurred between 1-4 weeks.

In the WHO database, joint ADRs constituted 1.3%, 0.6% and 2.9% of all ADRs for the 5-HT_{2A} blocking antidepressants, SSRIs and bisphosphonates, respectively. The difference between the 5-HT_{2A} antagonists and the SSRIs was significant ($p<0.001$), although the bisphosphonates had a significantly higher percentage of joint ADRs than the 5-HT_{2A} antagonists ($p=0.001$). However, when mianserin, alone, was compared with the bisphosphonates, there was no difference ($p=0.94$). Mianserin had also a significantly higher proportion of joint ADRs than either mirtazapine or nefazodone ($p<0.001$). The specific reaction term arthritis was also significantly more common for the 5-HT_{2A} antagonists compared with the SSRI group. This was also the case for mianserin and mirtazapine when taken alone, although not for nefazodone.

Joint ADRs in relation to DDD

When quotients were calculated between the ADR reports concerning joint disorders in the Swedish database and the DDDs for the corresponding

drugs, the 5-HT_{2A} antagonists had 45 times more joint ADRs reported than the SSRIs had ($p < 0.001$), whereas the reported joint ADRs of bisphosphonates were on the same level as for the 5-HT_{2A} antagonists.

Discussion

Inflammatory joint diseases such as RA have a complex etiology. Numerous of genetic and external risk factors have been identified. The impact of each new risk factor tends to only explain a smaller part of the etiology. Results from genetic and biochemical studies may also point out new markers for diseases, which sometimes may reflect causal relationships. Collection of epidemiological data may give signals about new external risk factors, and sometimes also contribute to the generation of hypotheses about the mechanistic background of diseases.

In study I data were presented that suggested an association between RA and low density of 5-HT_{2A} receptors. With respect to the results in study II, it is possible that at least in part this difference was genetically determined. The signal of joint symptoms as possible ADRs of 5-HT_{2A}-blocking atypical antidepressants, in study III, may give additional support to a hypothesis that a reduced number of 5-HT_{2A} receptors, available for binding, are significant as a new potential risk factor for joint disease, *e. g.*, RA. The possible mechanism behind this is unclear. The results in study IV, pointed to a possible link between glucocorticoids and 5-HT_{2A} receptor density that may have relevance in that context. In the following section possible mechanisms behind the results will be discussed as well as limitations of the studies.

Low 5-HT_{2A} receptor density in joint disease – cause or consequence?

The lower B_{max} values in RA patients found in study I could either represent a downregulation of 5-HT_{2A} receptors secondary to the rheumatic disease or be constitutional and thereby possibly contributing to an increased susceptibility to RA. With the first alternative the decreased 5-HT_{2A} receptor density must only be considered as an epiphenomenon, *i. e.*, a consequence of inflammation with no sense for the etiology of joint diseases or joint symptoms. A weak support for that idea is that a significant, but not strong, inverse correlation between B_{max} and ESR was found in RA patients in study I, although not in the controls. However, this finding was not replicated in PMR patients in study IV. There would even be a possibility that decreased levels of 5-HT_{2A} receptors were a physiological reactive downregulation aimed to manage the inflammation in the body. If this was true low levels of

5-HT_{2A} receptor density have to be considered as protecting from joint symptoms rather than the reverse. Anyway, the results from the genetic study (II) instead supported a hypothesis of a constitutionally lower 5-HT_{2A} density level in RA patients.

In study IV, an attempt to control for bias adherent to the status of platelets was performed using the TRC counts and the differences in TRC counts between baseline and follow-up visits, although no associations between B_{max} and these TRC values were found. However, as pointed out in Paper IV, a possible caution in the interpretation is necessary since it cannot be excluded that inflammatory diseases influence the proportion of different ages of the platelets. Different ages of platelets have been reported to constitute different subpopulations of platelets [185], which may differ in 5-HT_{2A} receptor status.

Other types of receptors and pathways that may influence 5-HT_{2A} density

The results found in studies I-III do not necessarily be interpreted as 5-HT_{2A} receptors having a direct impact on the pathophysiology of joint disease. The 5-HT_{2A} receptor may affect or be affected by another receptor or biochemical pathway with a more direct pathophysiological role. As in other biological pathways and receptor systems, the outcome of function of a certain receptor type, *e. g.*, the 5-HT_{2A} receptor, is the result of a complex regulation of several inhibitory and excitatory signals that come from other interconnected receptors of various types. Thus, in that context it is a disadvantage to only study 5-HT_{2A} receptors isolated from receptors that have been demonstrated to interact with HT_{2A} receptors, especially 5-HT_{1A} receptors [52, 186], noradrenergic receptors [62, 187-189] and dopaminergic receptors [190]. Such interactions may influence the results obtained in studies I and IV. It has been suggested that a down-regulation of HT_{2A} receptors may shift the balance between different types of serotonin receptors [191]. A quadratic relationship between the cerebral serotonin transporter (SERT) binding and 5-HT_{2A} receptor binding has also been reported [192].

The HPA axis is a further pathway that must be taken into account. The relationship between the HPA axis and the serotonin system has been shown to be bi-directional [73, 74]. It cannot be excluded that the glucocorticoid effect on 5-HT_{2A} receptor density plays a role in the pathophysiology of joints disease. In study IV, the exogenous glucocorticoid administration increased 5-HT_{2A} receptor density in man, as previously also shown in rats

[64-70]. Cortisone treatment significantly reduced the increased free serotonin levels that were found in RA patients [164]. This might also have normalised decreased levels of 5-HT_{2A} receptor density since serotonin has been demonstrated to downregulate 5-HT_{2A} receptors [193]. Anyway, there is a more substantiated explanation of the mechanism behind the effects of glucocorticoids on 5-HT_{2A} receptors. Increase of glucocorticoid receptor expression has been reported to increase 5-HT_{2A} receptor levels in the CNS of mice. This was reverted by glucocorticoid receptor blockade and was independent of 5-HT levels [194]. In another study 5-HT_{2A} receptors were suggested to be under the direct or indirect tonic inhibitory control of glucocorticoid receptors in rat CNS [195]. A further study reported that transcription of the HTR2A gene promoter was regulated by the glucocorticoid receptor [196].

Other diseases or conditions that may have significance for the relation between 5-HT_{2A} receptor density and RA

5-HT_{2A} receptors, psychiatric diseases and RA

There are several studies suggesting that the prevalence of depression occurs in higher frequency in patients with RA than in the general population. Although, the figures reported vary considerably among different studies, which partly may be due to differences between study populations and study design [197-200]. In contrast, many studies have demonstrated an inverse association between RA and schizophrenia [146-150]. Ligand binding studies performed on platelets of depressed patients [110-113] and of patients with schizophrenia [124-127] have shown increased density of 5-HT_{2A} receptors. A skewed distribution of psychiatric patients between the RA group and control group would have biased the results, but since psychiatric diseases were exclusion criteria, this should not be a big problem. However, it cannot be excluded that occasional individuals with minor subclinical psychiatric diseases might have been overlooked. The idea that 5-HT_{2A} density may have sense for the inverse association between RA and schizophrenia was consistent with the results of lower level of 5-HT_{2A} density in RA patients in study I and the previous studies showing increased density in schizophrenia. However, the results of lower rs6313 C allele frequency in both RA and schizophrenia deviated from this idea.

5-HT_{2A} receptors, fibromyalgia and RA

The prevalence of fibromyalgia has been shown to be higher in cohorts of RA patients [201, 202]. Fibromyalgia has been reported to have an inverse association to the TT-genotype of rs6313 [203]. Later two smaller studies gave no significant results [204, 205], although also in these two studies, the TT-genotype was less common among patients with fibromyalgia. Furthermore, a meta-analysis performed on the three referred studies found that the TT-genotype was significantly more uncommon in fibromyalgia patients as compared with controls [206]. Indeed, the same result as found in the RA cohorts in study II and summarised in table 3 above. In addition, the TT-genotype was associated with experience of higher pain severity [203] and lower pain threshold [204] in fibromyalgia. In contrast to this, a study on chronic widespread pain associated with fibromyalgia found an association between the rs6313 A allele (corresponding to the T allele) and a decrease in the total number of pain sites [207], although the association was not significant after controlling for symptoms of depression.

5-HT_{2A} receptors, cardiovascular disease and RA

The serotonin system is of importance for the cardiovascular system [208] and serotonin may be associated with coronary artery disease [209]. As mentioned above, 5-HT_{2A} receptors are present in vascular smooth muscle cells [32], *e. g.*, in the coronary arteries [208, 210]. Furthermore, platelet mediated vascular contractions have been reported to be inhibited by the 5-HT_{2A} receptor blocker ketanserin [211]. Interestingly, in connection to this, is the association between RA and cardiovascular disease found in many studies [212]. Two genetic association studies have been performed on the rs6313 SNP and myocardial infarction (MI). The first study with 255 patients found a significant association between non-fatal acute MI in Japan and the TT genotype [213], while a later study in 210 patients with acute MI in Spain found no significant association [214].

5-HT_{2A} receptors, cigarette smoking and RA

Cigarette smoking may be a possible confounder of the association found in study I between the RA disease and 5-HT_{2A} receptor density since one study found that 5HT_{2A} receptor density was higher among smokers and that there was a dose-dependent relationship between density and the quantity of

smoked cigarettes [215]. Cigarette smoking is an established risk factor for the development of RA. Since study I was not controlled for cigarette smoking, the RA patients may have possibly terminated previous smoking to a higher extent than did controls. However, another previous study found no effects of smoking habits on calcium responses to serotonin in platelets, which in turn showed a correlation to 5-HT_{2A} receptor density [106]. Some studies have found an association between HTR2A SNPs and cigarette smoking [216-218], while other studies did not [219, 220]. Furthermore, the two studies that found an association of smoking to rs6313 were contradictory in that the CC genotype was associated in one study while the TT genotype was associated in the other study [217, 218].

Possible mechanisms behind the results

In relation to a discussion of possible mechanisms underlying the results obtained in studies I-IV, the following question from paper I is of great relevance: “Are the changes restricted to platelets or does the 5-HT_{2A} receptor status in platelets reflect changes in some other type of cells or tissue which have importance for the pathophysiology of RA?” In the absence of further studies, a convincing answer could not be given. However, the following section will discuss some various possibilities concerning this subject. It is either possible that 5-HT_{2A} receptors in one certain tissue or cell type have significance for RA and joint symptoms, or that 5-HT_{2A} receptor-mediated effects on the pathophysiology of joint disease could be the result of an interaction of two or more cell types which express 5-HT_{2A} receptors.

Are the 5-HT_{2A} receptors on neurons of significance in joint disease?

The method using platelets to study 5-HT_{2A} receptor status as a model of neurons in the CNS had already been developed at our department from earlier for studies of an antidepressant drug [8]. In those studies 5-HT_{2A} receptors of CNS were of particular interest. Although not obvious, 5-HT_{2A} receptors in CNS may also be of interest for a study of this receptor in relation to joint disease. In any case central serotonergic mechanisms could not be excluded as a factor that influences the susceptibility to or severity of joint diseases. In a study where 5'-7'-dihydroxytryptamine, a neurotoxin causing serotonin depletion, was infused into the lateral CNS ventricle of adjuvant treated rats, a significant reduction of hind paw oedema was seen

[221]. That reduction was considered as greater than reduction of hind paw oedema in rats following total body depletion of serotonin using a tryptophan hydroxylase inhibitor [222]. Thus, it is possible that platelets, by reflecting 5-HT_{2A} receptor status in the CNS, show an association to RA. However, there are reasons to state that 5-HT_{2A} receptor status in platelets does not necessarily say anything about this status in other tissues. As noted above, the suggested correlation between 5-HT_{2A} receptor density in platelets and that in the CNS, which has been used for long in many studies, has been questioned in a study measuring the 5-HT_{2A} receptor density in platelets and in the CNS simultaneously, in normal healthy volunteers [104].

Another possibility is that 5-HT_{2A} receptors of more peripheral neurons have importance for joint diseases. In rats, increased activity in afferent nerve C-fibres induced by intra-articular serotonin has been found to be blocked by the 5-HT_{2A} receptor antagonist ketanserin [223]. Another study found 5-HT_{2A} receptor mRNA expressed in the lumbar spinal dorsal horn, and that the expression was significantly increased following carrageenan-induced inflammation [224]. Furthermore, stimulation of 5-HT_{2A/C} receptors with the 5-HT_{2A} receptor agonist DOI in the lumbar dorsal horn of rats potentiates the capsaicin-induced release of substance P [225]. Substance P has been implicated in the pathogenesis of RA [226-230]. However, another study showed that serotonin induced plasma extravasation of rat knee joints was not inhibited by C-fibre depletion, but by surgical destruction of lumbar nerve efferents. Additionally, this study found 5-HT_{2A} but not 5-HT_{2C} receptor mRNA present in lumbar sympathetic ganglia and furthermore that ketanserin blocked the plasma extravasation [159]. In addition, another study found that treatment with the 5-HT_{2A} antagonist, ritanserin, decreased arthritis in rats [160]. However, all these studies concerning 5-HT_{2A} receptors and peripheral neurons are based on the assumption that higher levels of 5-HT_{2A} receptors increasing the risk and degree of inflammation. This must primarily be considered as in contrast to the results of studies I-III.

Are the 5-HT_{2A} receptors on platelets of significance in joint disease?

Neither 5-HT_{2A} receptor status in neurons, nor the suggested correlation between 5-HT_{2A} density in platelets and 5-HT_{2A} density in the CNS, are conditions of importance to the interpretation of the shown relation between 5-HT_{2A} receptors and RA. In fact, some earlier studies concerning RA have focused on, precisely, the impact platelets may have on the disease, although not specifically platelet 5-HT_{2A} receptors [163, 166, 231-233]. In addition, platelets have recently been attributed with an active, critical role in the

pathogenesis of inflammation in rheumatoid arthritis [234]. In murine experiments, it has been demonstrated that platelets enhance vascular permeability during autoimmune inflammatory arthritis through the formation of interendothelial cell gaps in joints. This increased formation was mediated by the release of serotonin from platelets [235]. Although it has yet to be proven, it may be possible that this serotonin release could be mediated, in part, through 5-HT_{2A} receptors.

Are the 5-HT_{2A} receptors on other cell types of significance in joint disease?

Neurons and platelets are, however, not the only possible candidates for 5-HT_{2A} receptor mediated effects on joints. Some additional alternatives should be mentioned below. Immune cells, especially T-lymphocytes (T-cells), are strong candidates since T-cells are considered to play a key role in RA. Neither in this project nor in earlier studies of ligand binding in lymphocytes there have been convincingly findings of specific binding of 5-HT_{2A} receptors [236]. Nevertheless, HTR2A mRNA has been detected in both human and rodent lymphocytes [36, 38, 93] . In addition, 5-HT_{2A} receptors have been detected with immunostaining on human CD3+ cells [237], and 5-HT_{2A} receptor protein has been demonstrated with western blot within CD4+ and CD8+ T-cells from mice [238]. Furthermore, there are a few functional studies which may provide further evidence for the existence of 5-HT_{2A} receptors on lymphocytes: three studies performed in mice showed that there was 5-HT_{2A} receptor-mediated enhancement of lymphocyte activation and production of inflammatory cytokines, *e. g.*, IFN- γ and IL-2 [238-240]. A fourth study, performed *in vitro* on human T-cells, demonstrated that both serotonin, and ketanserin in high concentration, inhibited a chemical induced proliferative response of lymphocytes, while lower concentrations of ketanserin instead inhibited the anti-proliferative effect of serotonin [241].

HTR2A mRNA has also been detected in macrophage-like synovial cells [242], and it has been shown that the 5-HT_{2A} receptor antagonist ketanserin blocked the inhibitory effect of serotonin on TNF- α synthesis of human monocytes [243]. Furthermore, 5-HT_{2A} receptors have been detected in vascular smooth muscle cells [32], and stimulation of 5-HT_{2A} receptors has been shown to highly suppress TNF- α induced inflammation in rat aortic smooth muscle cells [244]. In contrast, HTR2A mRNA was not found in human endothelial cells [245]. It has also been reported that synovial fibroblasts express 5-HT_{2A} receptors [246].

What is the effect on joints of 5-HT_{2A} antagonism and of decreased levels of 5-HT_{2A} receptors?

The increased level of joint ADR reports connected to the treatment with atypical 5-HT_{2A} blocking antidepressants found in study III was in line with the results of studies I and II which showed decreased 5-HT_{2A} receptor density and a lower level of rs6313 TT genotype. The results of study III was furthermore in line with previous reports of joint symptoms as ADRs of atypical 5-HT_{2A} blocking antidepressants [247-250]. Interestingly, it concurred also with an old study where local treatment with intra-articular injections of the partial 5-HT_{2A} agonist methysergide were tested in 27 patients with a joint disease, 22 of them had RA, four with osteoarthritis and one with infection-related joint disease. In seven cases, all with RA, there was a definite improvement and in four patients there was a slight improvement [168]. It is possibly also in line with a study that showed increased plasma levels of TNF- α following four weeks of treatment with mirtazapine in patients with depression [251]. In paper II it was suggested that serotonin reuptake inhibition, resulting in an increased level of serotonin in the synaptic cleft, and hence increased stimulation of 5-HT_{2A} receptors, may be a possible new therapeutic option in RA. In two recent studies, SSRIs were found to be effective in the treatment of experimental arthritis in rodents. In both of these studies the TNF- α levels were decreased by the treatment [161, 162].

From the perspective that serotonin is considered to have pro-inflammatory effects, and from the perspective that the 5-HT_{2A} receptor is thought to mediate excitatory signals when serotonin binds to the receptor, the results found in study I-III were unexpected. In view of the results which showed improvement of arthritis in rats following treatment with the two 5-HT_{2A} antagonists ketanserin and ritanserin [159, 160] it would rather have been expected that there would be a finding of a higher average 5-HT_{2A} receptor density in RA patients and a lower level of joint ADR reports from those taking 5-HT_{2A} blocking antidepressants. What could account for these conflicting results? Assuming that all these studies are valid various hypotheses to explain the contradictory results can be listed. Firstly, the studies performed in rats were experimental and do not, with any certainty, reflect the often far more complex pathogenic mechanisms in human patients. As is known from other fields in medicine, even well constructed animal models do not always correspond to human diseases [252-254]. As mentioned above, there are differences between the human 5-HT_{2A} receptor and the corresponding 5-HT_{2A} receptor in rats. Although these differences are small they have been demonstrated as leading to differences in the pharmacological properties of the receptor [54, 78, 255]. In addition,

differences between species with regard to serotonin as a mediator of vascular permeability have been previously described [256], as well as differences in the function of serotonin as a mediator of vascular permeability between healthy and diseased blood vessels [257, 258]. Secondly, the acute effects of stimulation of a receptor with a certain drug are not necessarily identical to the effects of chronic stimulation with the same drug. The 5-HT_{2A} blocking antipsychotic drug clozapine has, for example, been reported to cause an increase in 5-HT_{2A} mRNA levels in the CNS of rats after four days, but this was not seen after 32 days of treatment [259]. Thirdly, many different pathways appear to be linked to the 5-HT_{2A} receptor, and it is not easy to conclude what the net effect of increased signalling or inhibition of these pathways will be. Even if stimulation of 5-HT_{2A} receptors causes a neuronal contribution to the susceptibility or severity of a joint disorder, 5-HT_{2A} mediated signalling on other pathways, e. g., suppression of TNF- α secretion, may overweigh. Furthermore, one study reported that stimulation of 5-HT₂ receptors may either increase or decrease TNF- α production depending on the actual levels of serotonin. Physiological levels of serotonin did not influence TNF- α levels, whilst higher concentrations of serotonin and the serotonin depleting agent PCPA both suppressed TNF- α production [260].

Are 5-HT_{2A} receptors of significance in joint disorders other than RA and joint ADRs?

A further question raised regarding the results of study I was whether the changes in 5-HT_{2A} receptor B_{max} were restricted to RA, or also could be seen in other rheumatic or autoimmune diseases. To date, there has been no other study published where patients with an inflammatory joint disease have been compared to controls with respect to 5-HT_{2A} receptor density. The lack of a control group in study IV makes it difficult to conclude anything about 5-HT_{2A} receptor density in PMR and GCA patients. The findings in other studies that the CC genotype of rs6313 is more common in patients with fibromyalgia or temporomandibular dysfunction, may have some relevance to this field [206, 261]. However, one must keep in mind that there is a possibility that this receptor does not have any importance for joint diseases or autoimmune diseases in general, but is only linked to a pathophysiological mechanism that is specific to RA.

Limitations and possible bias of the studies

Since these are the first human studies in this field, further studies are needed to confirm the findings. There are many limitations and possible biases inherent with the present studies, which may lead to incorrect conclusions. Some of these limitations and possible bias will be discussed below.

Limitations regarding the cohorts used

Well-matched control groups are a condition for a valid comparison and reliable results. The control group used in study I is probably one of the most well-matched and controlled 5-HT_{2A} receptor binding studies that has ever been undertaken. However, there are some possible factors that may cause bias. It is possible that the tendency to move to or from different geographical regions may have differed depending on whether or not a person has RA. Possible differences in genetically constitution for the HTR2A gene between different geographical regions may then influence the results since there was no controlling for birthplace in the study. However, urban/rural differences in RA have been explained by age differences which were controlled for in study I [134]. The study was not either controlled for smoking habits, which may influence 5-HT_{2A} receptor density [215].

It would have been tempting to use the mean B_{\max} for control groups from other studies to assess the reliability of study I as well as to make a comparison with the PMR/GCA patients in study IV. However, this would not be advisable since the method is not standardized. Previous studies using the same ligands and method have yielded great differences in the mean of B_{\max} for control groups making comparisons between control groups across different studies difficult. This applies even in some cases to studies which have been performed at the same laboratory [262, 263]. There are some possible reasons for this, besides the possibility of true differences between control groups caused by factors such as ethnicity or sunlight exposure. Firstly, the rate of decomposition of the [³H]LSD is approximately 4% per month, which contributes to a lot-to-lot variation in radioactivity. Secondly, it is possible although not reported, that receptor protein degradation before analyses may occur and contribute to variations in the results of analyses. Thirdly, small differences in the practical handling of the binding experiments could possible affect the results.

The controls used in the discovery phase of study II consisted indeed of three different control groups, none of which was matched to the RA patient group on an individual level. Although none of these control groups were designated for the actual RA patient group, they were from the same geographic region and the female/male ratio appeared to be almost the same, *i. e.*, 2.435 in the RA group versus 2.425 in the control group. In contrast, controls in the EIRA study had a female/male ratio of 2.96, which was rather higher than their corresponding patient group, which had a ratio of 2.43. However, since there were no significant differences in genotype or allele frequencies between sexes this may be reasonable to accept. Even though the frequencies of genotypes and alleles differed significantly between the RA groups and control groups, respectively, in the discovery phase compared with the validation phase, all separate groups were in Hardy-Weinberg equilibrium. The differences in frequencies between the two phases may be by chance, but since results from previous studies indicate that rs6313 genotype frequencies varies between different geographical regions even for control groups [264], it may also mirror differences in population genetics between Northern and Southern Sweden [265]. The differences in rs6313 frequencies were most pronounced when comparing the two RA groups. One difference between these two RA cohorts is that whilst the majority of the patients from Northern Sweden had been first diagnosed with the disease many years ago, the EIRA cohort consisted of patients who had recently been diagnosed. The latter fact may increase the risk that some individuals included may appear to not fulfil the criteria for RA later and have to be re-diagnosed with another disease.

Limitations regarding the genetic studies

It cannot be excluded that the associations between RA and the genotypes, rs6311, rs6313, rs6314 and rs1328674, while significant, were merely random. After a test for multiple comparisons there was only a weak significance for allele frequencies of rs1328674. However, it is possible that the study could inherit type 2 errors if each SNP has a small true association to RA which needs a very large number of individuals to be detected. Nevertheless, the association of the rs6313 and RA was shown in two different cohorts (study II). This is a reason why one can question whether it was correct to include rs6313 in the test for multiple comparisons.

It is also possible that the impact of variation in HTR2A SNPs on susceptibility to the disease differs among various RA populations and among different pathophysiological subtypes of the disease. This is possibly

the case regarding the relation between HTR2A and schizophrenia, where an association to rs6313 seems more likely in European cohorts than in Asian cohorts [123, 133], as well as in patients responding to the neuroleptic drug clozapine [266]. One factor that may have explanatory impact for regional differences is the different degree of exposure to daylight in different regions. Since the density of 5-HT_{2A} receptors has been reported to be lower in summer and winter in Northern Sweden [176], it is possible that patients with in mean lower density of 5-HT_{2A} receptors due to a rs6313 CC genotype are more susceptible to RA, if they live in this area.

In addition, to the rather weak association between genotypes and alleles of the HTR2A gene and RA, two haplotypes constructed of the four genotypes, rs6311, rs6313, rs6314 and rs1328674, showed a quite strong negative and positive association, respectively, to RA, with the TCTT considered as a protective haplotype against RA, and the mirror combination CTCC, as susceptible for RA. However, the construction of these haplotypes may be questionable. Firstly, rs6311 and rs6313 have been described in studies to be in complete linkage disequilibrium (LD) [207]. However, not all studies do support a strong LD between these two SNPs [123]. Secondly, RA patients tended to associate with the His452His variant of rs6314. This variant has in expression studies shown better 5-HT_{2A} receptor function than the His452Tyr variant [86, 87]. This is in contrast to the results of rs6313 where RA patients associated with the CC genotype, which expression studies have reported as being associated with lower expression of receptors [90-92].

After the initiation of the studies I and II the concept of epigenetics has become more generally established [267, 268]. In the light of knowledge from this field it is tempting to suggest that, besides genetic determined levels of 5-HT_{2A} receptor density, epigenetic factors could account for a further part of the differences in 5-HT_{2A} receptor B_{max} between RA patients and controls in study I. This has also been proposed in relation to the above-mentioned possible association between the HTR2A gene and schizophrenia [269-271]. It has been reported that the mRNA expression from the C-allele in the rs6313 SNP is lower than expected [92]. For these reasons, an analysis is included, in table 3 in this dissertation book, where only the frequencies of CC and TT genotypes of the cohorts in study II were compared. The monoallelic expression may be more pronounced in certain tissues or cell types, *e. g.*, demonstrated for peripheral blood lymphocytes [93], although another study did not replicate that finding in peripheral blood leukocytes [272]. The mRNA expression has also been shown to be different between CNS and platelets of the same individual [99]. In addition, the monoallelic expression is reported to occur only in some individuals [273]. The

monoallelic expression seems not to be a consequence of genomic imprinting [93, 272].

Limitations with the epidemiologic study

Incidences are based on some type of ratio. In the case of the two databases used in study III the large degree of underreporting made the numerator of this ratio uncertain. Underreporting is in general common [274] and may contribute to the risk of type 2 errors or at least an underestimation of the size of a true association. Furthermore, since it was not known how many persons were treated with different drugs, the denominator was also uncertain or even lacking, when dealing with these ADR databases. The lack of an evident denominator makes it difficult to do robust estimates of the incidence of a possible ADR and also difficult to do a comparison with the normal incidence of the same symptom or disease in a population. The statistical approach of relating joint ADRs to all ADRs may have led to bias, since this incidence ratio for joint ADRs will to some extent depend on the tendency to report other types of ADRs. High reporting frequencies of other types of ADRs would have diminished a signal of joint ADRs, whilst low reporting frequencies of other types of ADRs would have increased the ratio, which may have led to a false signal of joint ADRs. Since neither the frequency of the studied joint symptoms in a normal population nor in a depressed population were known, it could not be excluded that the SSRI substances had a protective effect against joint disorders, thereby producing a false positive signal of 5-HT_{2A} blocking antidepressants as having an increased level of joint ADRs.

The other approach of relating ADRs to sold DDDs may lead to another source of bias. The general tendency to report ADRs may vary among different drugs and different groups of drugs, and this will influence the figure level of all ratios between ADRs and DDD. Another possible bias when it comes to relating an ADR to the sales figures (DDD) is that the extent to which the drugs were actually used by the patients was unknown. The non-adherence to prescription of antidepressants has been reported to be high [275]. Another potential bias concerning the sales figures is that DDD has not always been shown to be consistent with prescribed daily doses [276]. When considering information of established DDD for the antidepressants included in study III (provided in table 2) it seems obvious that while the DDD figure for mianserin corresponds rather well to commonly prescribed doses, some of the SSRIs probably have lower DDD than commonly prescribed doses. However, a study that compared different

dose standard units found that DDD was the most appropriate unit for pharmacoepidemiological studies [277].

The ADR databases have since their start had a different diagnosis classification system and sometimes different diagnosis criteria than otherwise commonly used classification systems and criteria, such as the ICD classification system. This deficiency in comparability between the diagnosis classification systems makes it sometimes difficult to know how to assess the various ADR reports included in study III. Merging the two ADR terms arthritis and arthralgia was not an uncomplicated matter. Arthralgia may sometimes be related to arthritis, although in most cases not. However, regarding suspected ADRs of the same drugs, it may be reasonable to study these terms together.

Confounding by indication is a possible source of bias in pharmacoepidemiological studies. It has been suggested that atypical 5-HT_{2A} blocking antidepressants may have been prescribed in a greater extent than SSRI to depressed patients experiencing chronic pain which possibly could be accompanied by joint symptoms and sleep problems. Since atypical 5-HT_{2A} blocking antidepressants have a positive effect on sleep continuity, it therefore may be plausible that prescribers more often have chosen to prescribe these drugs to that type of patients [278]. This argument is clearly warranted. However, the vast majority of the reports from the Swedish ADR database included information of the time interval between start of treatment and the appearance of the joint symptoms, *i.e.*, medication with the antidepressants preceding joint symptoms rather than the reverse.

The relatively high level of joint ADR reports concerning 5-HT_{2A} antagonists, and especially mianserin, could at least in part be due to reporting bias caused by the fact that there already were published articles describing case reports or case series of patients with suspected joint ADRs during treatment with atypical 5-HT_{2A} blocking antidepressants. Contrary reporting bias also may go in the other direction, showing a reduced number of reported ADRs following the publishing of articles about the same ADR. The latter would be due to the prescribers thinking that the ADR is already a known fact, and no longer needs to be reported, and this is especially the case if the ADR is not a serious one. The call from MPA to report all ADRs for new drugs, and only all serious ADRs for all other drugs may have resulted in many joint ADRs never being recognised and even less being reported.

It should be noted that we did not know anything about 5-HT_{2A} receptor density in the patients who experienced the joint symptoms described in the

ADR reports of study III. Most studies have reported lower density following mianserin administration [51, 55-57, 279, 280], although the only study performed specifically in human platelets, *in vivo*, demonstrated an upregulation of 5-HT_{2A} density in five individuals after five days of treatment with mianserin [58]. However, this was a very small human study with the perspective of the known big intraindividual variability in 5-HT_{2A} receptor density. Furthermore, and most importantly, mianserin-induced downregulation has been suggested as at least in part, resulting from the ability of modulating transcription [193], which means that a treatment period of five days might be too short time to detect the pronounced downregulation of 5-HT_{2A} density found in other studies. In this context, some remarks concerning regulation of 5-HT_{2A} receptors caused by medication will be mentioned. In study IV, the increase in platelet 5-HT_{2A} receptor B_{max} from the start of treatment with prednisolone to the last follow-up visit, after an average of 33.6 days, was 43.8 %, and significant. [64-70, 75]. The percentage increases in the mean CNS B_{max} were, in previous animal studies which lasted for at most two weeks (*i.e.*, between 7 and 14 days), within the range of between 2.5% and 28%. In that range was the 13.8% increase in the mean B_{max} from samples drawn at the first follow-up visit of study IV after an average of 8.8 days. In conclusion, the final result of regulation of 5-HT_{2A} receptors by subchronic or chronic medication probably needs at least 1-2 weeks to get established. In paper III the median of appearance of joint ADRs was 21 days, and 50 % occurred between 1-4 weeks.

Great possibilities for bias are obvious in studies based on so-called spontaneous reporting. The advantage of using such large database as that of the WHO ADR database may be that randomly false positive findings will hence be easier to avoid. Using data regarding ADRs from a database similar to the Swedish one where a validation of the suspected ADRs is provided together with clinical data related to the adverse events, may also increase the reliability of the study. The approach of studying the suspected ADRs in two different ways, *i. e.*, related to all ADRs for the drug and to sold DDDs for the drug, respectively, may further increase reliability. Despite the many potential disadvantages in the present epidemiological study listed above, the study may nevertheless provide a sufficiently strong signal so as to warrant further studies. Although the main benefit of the study was to generate a signal of joint symptoms as a possible ADR of atypical 5-HT_{2A} blocking antidepressants and although demonstrating causality are not possible with this type of studies, this study nevertheless strengthens the signal of joint symptoms as ADRs of atypical 5-HT_{2A} blocking antidepressants more than do isolated case reports or case series.

Concluding remarks

The studies included in this thesis have indicated that the 5-HT_{2A} receptor is of significance in joint disease. Density of 5-HT_{2A} receptor protein was found to be lower in RA patients and may be a new possible etiological factor and risk marker for RA. This lower density may be, in part, genetically determined. External factors that cause a decrease in 5-HT_{2A} density or signalling through 5-HT_{2A} receptors, *e. g.*, atypical 5-HT_{2A} blocking antidepressants, may also increase the risk of developing a joint disease. The mechanisms behind a possible increased risk of joint disease in relation to low 5-HT_{2A} receptor density are unclear, although interactions between serum glucocorticoid level and 5-HT_{2A} receptors may play a role.

Conclusions

The density of 5-HT_{2A} receptor protein was significantly lower in patients with rheumatoid arthritis from Northern Sweden when compared to age and sex matched controls, while the affinity of 5-HT_{2A} receptors was similar to that of the controls.

Rheumatoid arthritis was associated with variations in four single nucleotide polymorphisms in the HTR2A gene. The association with one of these variations was demonstrated in two different Swedish cohorts.

Spontaneous reports of adverse drug reactions concerning joint disorders were more common following the use of atypical 5-HT_{2A} blocking antidepressants than selective serotonin reuptake inhibitors, relative to either sales figures or to all reports of adverse drug reactions for the same drugs.

The density of 5-HT_{2A} receptor protein in man was increased following administration of exogenous glucocorticoids.

Acknowledgements

I would like to thank:

My wife Maria for all love, patience and encouragement you have shown me, despite that you have had to wait for me so many times because of these studies and the thesis. Also for invaluable help with Excel and how to evaluate laboratory data and for layout.

My lovely children Hanna, Elina and David for all joy, and for that you have taught me so much about what is important in life.

My parents, for always helping me with different things and for that you have encouraged me to study from early childhood. You have always shown that you love me, regardless of my success or failure.

My sister Maria and my nieces Ingrid and Astrid, because we always have a good time when we meet.

My three supervisors Solbritt, Rune and Tom. You have accompanied me during my PhD project and given me many good advices. You have also made available resources for my research and shown me a positive and supporting attitude, despite my high-flying plans into previous unknown high-risk research from the perspective of chances to get useful results in relation to invested time and money.

The research- and ADR-nurses Marit and Martin for your help with recruitment of patients, for blood sampling and discussions concerning ADRs.

Ingrid Persson and Solveig Linghult for analysing many of the samples used in the studies, and for all discussions with me concerning the methods.

Jonas, because you have given me opportunity to be unoccupied of clinical duty and given me time to concentrate on this thesis. You have also supported me during difficult periods.

Roland, for your help with varying everyday practical things, and for discussions about various topics like drugs, cars and politics.

The current and former clinical pharmacologists in Umeå Bertil, Jörn, Staffan and Ulrika, who I have shared many interesting discussions and intellectual work together with.

Current and former employees at Klinisk farmakologi/Läkemedelscentrum and GKM for a good workplace.

Leonid Padyukov for interesting discussions of human genetics.

All my co-authors.

Olav Spigset for developing the method of [³H]LSD binding in Umeå many years ago.

The staff who have helped me with different things at the Rheumatology clinic.

All patients and controls included in the studies.

The persons I have worked together with at the former unit KUB and at the present group for AT/ST issues for being positive to my attempt to finish this thesis.

The former professor Andrej Tarkowski who raised my interest for scientific questions in relation to rheumatology during my time of clinical duty in Gothenburg.

Henric, Kjell-Ola and all my friends, for prayers, support and good fellowship.

The funds, Insamlingsstiftelsen and Arnerska fonden at Umeå University, ALF at Västerbottens Läns Landsting, and the Foundation for Clinical Pharmacology and Pharmacy in Sweden, for economical support to the project.

Finally, I want to give my very greatest thanks to my God, the Giver of all good gifts, who has given me meaningfulness in my work and the opportunity to know all the wonderful people mentioned above.

Thanks be to God for His indescribable gift! (2 Corinthians 9:15)

References

1. Berger M, Gray JA, Roth BL. The expanded biology of serotonin. *Annu Rev Med.* 2009; 60:355-366.
2. Erspamer V, Asero B. Identification of enteramine, the specific hormone of the enterochromaffin cell system, as 5-hydroxytryptamine. *Nature.* 1952 May 10; 169(4306):800-801.
3. Feldberg W, Toh CC. Distribution of 5-hydroxytryptamine (serotonin, enteramine) in the wall of the digestive tract. *J Physiol.* 1953 Feb 27; 119(2-3):352-362.
4. Rapport MM, Green AA, Page IH. Serum vasoconstrictor, serotonin; isolation and characterization. *J Biol Chem.* 1948 Dec; 176(3):1243-1251.
5. Sanders-Bush E, Hazelwood L. Goodman & Gilman's the pharmacological basis of therapeutics [Elektronisk resurs]. In: Brunton L, ed. *Chapter 13 5-Hydroxytryptamine (Serotonin) and Dopamine.* 12th Edition ed 2012.
6. Brodie BB, Pletscher A, Shore PA. Evidence that serotonin has a role in brain function. *Science.* 1955 Nov 18; 122(3177):968.
7. Hardisty RM, Stacey RS. 5-Hydroxytryptamine in normal human platelets. *J Physiol.* 1955 Dec 29; 130(3):711-720.
8. Spigset O. The selective serotonin reuptake inhibitor fluvoxamine : aspects on pharmacokinetics, pharmacodynamics and adverse effects in healthy volunteers. Umeå: Univ., 1997.
9. Kim DY, Camilleri M. Serotonin: a mediator of the brain-gut connection. *Am J Gastroenterol.* 2000; 95(10):2698-2709.
10. Steiner JA, Carneiro AM, Blakely RD. Going with the flow: trafficking-dependent and -independent regulation of serotonin transport. *Traffic.* 2008; 9(9):1393-1402.
11. Gaddum JH, Picarelli ZP. Two kinds of tryptamine receptor. *Br J Pharmacol Chemother.* 1957 Sep; 12(3):323-328.

12. Peroutka SJ, Snyder SH. Multiple serotonin receptors: differential binding of [3H]5-hydroxytryptamine, [3H]lysergic acid diethylamide and [3H]spiropiperidol. *Mol Pharmacol*. 1979 Nov; 16(3):687-699.
13. Sharman JL MC, Spedding M, Germain P, Staels B, Dacquet C, Laudet V, Harmar AJ, and NC-IUPHAR. (2011), data. I-Dnratesavop, D534-D538. *NARDI*. [cited 2012-05-21]; Available from: <http://www.iuphar-db.org>
14. Hoyer D, Hannon JP, Martin GR. Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol Biochem Behav*. 2002 Apr; 71(4):533-554.
15. Humphrey PP, Hartig P, Hoyer D. A proposed new nomenclature for 5-HT receptors. *Trends Pharmacol Sci*. 1993 Jun; 14(6):233-236.
16. Bockaert J, Claeysen S, Becamel C, Dumuis A, Marin P. Neuronal 5-HT metabotropic receptors: fine-tuning of their structure, signaling, and roles in synaptic modulation. *Cell Tissue Res*. 2006 Nov; 326(2):553-572.
17. Palacios JM, Waeber C, Hoyer D, Mengod G. Distribution of serotonin receptors. *Ann N Y Acad Sci*. 1990; 600:36-52.
18. Harker LA, Slichter SJ. Platelet and fibrinogen consumption in man. *N Engl J Med*. 1972 Nov 16; 287(20):999-1005.
19. Vanhoutte PM. Vascular effects of serotonin and ischemia. *J Cardiovasc Pharmacol*. 1990; 16 Suppl 3:S15-19.
20. Mercado CP, Kilic F. Molecular mechanisms of SERT in platelets: regulation of plasma serotonin levels. *Mol Interv*. 2010; 10(4):231-241.
21. Pletscher A. Platelets as models: use and limitations. *Experientia*. 1988 Feb 15; 44(2):152-155.
22. Loric S, Launay JM, Colas JF, Maroteaux L. New mouse 5-HT₂-like receptor. Expression in brain, heart and intestine. *FEBS Lett*. 1992; 312(2-3):203-207.

23. McKenna DJ, Peroutka SJ. Differentiation of 5-hydroxytryptamine₂ receptor subtypes using 125I-R-(-)2,5-dimethoxy-4-iodo-phenylisopropylamine and 3H-ketanserin. *J Neurosci*. 1989 Oct; 9(10):3482-3490.
24. Berg KA, Clarke WP, Sailstad C, Saltzman A, Maayani S. Signal transduction differences between 5-hydroxytryptamine type 2A and type 2C receptor systems. *Mol Pharmacol*. 1994 Sep; 46(3):477-484.
25. Leysen JE. 5-HT₂ receptors. *Curr Drug Targets CNS Neurol Disord*. 2004 Feb; 3(1):11-26.
26. Raote I, Bhattacharya A, Panicker M. Serotonin 2A (5-HT_{2A}) Receptor Function: Ligand-Dependent Mechanisms and Pathways. *Serotonin Receptors in Neurobiology*. 2007.
27. Conn PJ, Sanders-Bush E. Regulation of serotonin-stimulated phosphoinositide hydrolysis: relation to the serotonin 5-HT-2 binding site. *J Neurosci*. 1986 Dec; 6(12):3669-3675.
28. Pazos A, Probst A, Palacios JM. Serotonin receptors in the human brain--IV. Autoradiographic mapping of serotonin-2 receptors. *Neuroscience*. 1987 Apr; 21(1):123-139.
29. Jakab RL, Goldman-Rakic PS. 5-Hydroxytryptamine_{2A} serotonin receptors in the primate cerebral cortex: possible site of action of hallucinogenic and antipsychotic drugs in pyramidal cell apical dendrites. *Proc Natl Acad Sci U S A*. 1998 Jan 20; 95(2):735-740.
30. Carlton SM, Coggeshall RE. Immunohistochemical localization of 5-HT_{2A} receptors in peripheral sensory axons in rat glabrous skin. *Brain Res*. 1997 Jul 25; 763(2):271-275.
31. de Clerck F, David JL, Janssen PA. Inhibition of 5-hydroxytryptamine-induced and -amplified human platelet aggregation by ketanserin (R 41 468), a selective 5-HT₂-receptor antagonist. *Agents Actions*. 1982 Jul; 12(3):388-397.
32. Cohen ML, Fuller RW, Wiley KS. Evidence for 5-HT₂ receptors mediating contraction in vascular smooth muscle. *J Pharmacol Exp Ther*. 1981 Aug; 218(2):421-425.

33. Bai Y, Zhang M, Sanderson MJ. Contractility and Ca²⁺ signaling of smooth muscle cells in different generations of mouse airways. *Am J Respir Cell Mol Biol*. 2007; 36(1):122-130.
34. Fiorica-Howells E, Hen R, Gingrich J, Li Z, Gershon MD. 5-HT(2A) receptors: location and functional analysis in intestines of wild-type and 5-HT(2A) knockout mice. *Am J Physiol Gastrointest Liver Physiol*. 2002 May; 282(5):G877-893.
35. Mialet-Perez J, D'Angelo R, Villeneuve C, Ordener C, Negre-Salvayre A, Parini A, et al. Serotonin 5-HT_{2A} receptor-mediated hypertrophy is negatively regulated by caveolin-3 in cardiomyoblasts and neonatal cardiomyocytes. *J Mol Cell Cardiol*. 2012; 52(2):502-510.
36. Stefulj J, Jernej B, Cicin-Sain L, Rinner I, Schauenstein K. mRNA expression of serotonin receptors in cells of the immune tissues of the rat. *Brain Behav Immun*. 2000 Sep; 14(3):219-224.
37. Yang GB, Qiu CL, Zhao H, Liu Q, Shao Y. Expression of mRNA for multiple serotonin (5-HT) receptor types/subtypes by the peripheral blood mononuclear cells of rhesus macaques. *J Neuroimmunol*. 2006; 178(1-2):24-29.
38. Leon-Ponte M, Ahern GP, O'Connell PJ. Serotonin provides an accessory signal to enhance T-cell activation by signaling through the 5-HT₇ receptor. *Blood*. 2007 Apr 15; 109(8):3139-3146.
39. Akin D, Manier DH, Sanders-Bush E, Shelton RC. Decreased serotonin 5-HT_{2A} receptor-stimulated phosphoinositide signaling in fibroblasts from melancholic depressed patients. *Neuropsychopharmacology*. 2004; 29(11):2081-2087.
40. Wilcox BD, Rydelek-Fitzgerald L, Jeffrey JJ. Regulation of collagenase gene expression by serotonin and progesterone in rat uterine smooth muscle cells. *J Biol Chem*. 1992 Oct 15; 267(29):20752-20757.
41. Guillet-Deniau I, Burnol AF, Girard J. Identification and localization of a skeletal muscle serotonin 5-HT_{2A} receptor coupled to the Jak/STAT pathway. *J Biol Chem*. 1997 Jun 6; 272(23):14825-14829.
42. Sharif NA, Kelly CR, Crider JY, Davis TL. Serotonin-2 (5-HT₂) receptor-mediated signal transduction in human ciliary muscle cells: role in ocular hypotension. *J Ocul Pharmacol Ther*. 2006 Dec; 22(6):389-401.

43. Rasbach KA, Funk JA, Jayavelu T, Green PT, Schnellmann RG. 5-hydroxytryptamine receptor stimulation of mitochondrial biogenesis. *J Pharmacol Exp Ther*. 2010; 332(2):632-639.
44. Messori E, Rizzi CA, Candura SM, Lucchelli A, Balestra B, Tonini M. 5-Hydroxytryptamine receptors that facilitate excitatory neuromuscular transmission in the guinea-pig isolated detrusor muscle. *Br J Pharmacol*. 1995 Jun; 115(4):677-683.
45. Sommer C. Serotonin in pain and analgesia: actions in the periphery. *Mol Neurobiol*. 2004; 30(2):117-125.
46. De Clerck F, van Nueten JM, Reneman RS. Platelet-vessel wall interactions: implication of 5-hydroxytryptamine. A review. *Agents Actions*. 1984 Dec; 15(5-6):612-626.
47. Da Prada M, Cesura AM, Launay JM, Richards JG. Platelets as a model for neurones? *Experientia*. 1988 Feb 15; 44(2):115-126.
48. Aloyo VJ, Berg KA, Spampinato U, Clarke WP, Harvey JA. Current status of inverse agonism at serotonin_{2A} (5-HT_{2A}) and 5-HT_{2C} receptors. *Pharmacol Ther*. 2009 Feb; 121(2):160-173.
49. Berg KA, Harvey JA, Spampinato U, Clarke WP. Physiological relevance of constitutive activity of 5-HT_{2A} and 5-HT_{2C} receptors. *Trends Pharmacol Sci*. 2005 Dec; 26(12):625-630.
50. Wohlschlag KL, Molinoff PB. Regulation of levels of 5-HT_{2A} receptor mRNA. *Ann N Y Acad Sci*. 1998 Dec 15; 861:128-135.
51. Toth M. Transcriptional regulation of the 5-HT_{2A} receptor. *Behav Brain Res*. 1996; 73(1-2):183-186.
52. Carrasco GA, Van de Kar LD, Jia C, Xu H, Chen Z, Chadda R, et al. Single exposure to a serotonin 1A receptor agonist, (+)8-hydroxy-2-(di-n-propylamino)-tetralin, produces a prolonged heterologous desensitization of serotonin 2A receptors in neuroendocrine neurons in vivo. *J Pharmacol Exp Ther*. 2007 Mar; 320(3):1078-1086.
53. Gray JA, Roth BL. Paradoxical trafficking and regulation of 5-HT(2A) receptors by agonists and antagonists. *Brain Res Bull*. 2001 Nov 15; 56(5):441-451.

54. Roth BL, Willins DL, Kristiansen K, Kroeze WK. 5-Hydroxytryptamine₂-family receptors (5-hydroxytryptamine_{2A}, 5-hydroxytryptamine_{2B}, 5-hydroxytryptamine_{2C}): where structure meets function. *Pharmacol Ther.* 1998 Sep; 79(3):231-257.
55. Newton RA, Elliott JM. Mianserin-induced down-regulation of human 5-hydroxytryptamine_{2A} and 5-hydroxytryptamine_{2C} receptors stably expressed in the human neuroblastoma cell line SH-SY5Y. *J Neurochem.* 1997 Sep; 69(3):1031-1038.
56. Anji A, Kumari M, Sullivan Hanley NR, Bryan GL, Hensler JG. Regulation of 5-HT(2A) receptor mRNA levels and binding sites in rat frontal cortex by the agonist DOI and the antagonist mianserin. *Neuropharmacology.* 2000 Aug 23; 39(11):1996-2005.
57. Blackshear MA, Sanders-Bush E. Serotonin receptor sensitivity after acute and chronic treatment with mianserin. *J Pharmacol Exp Ther.* 1982 May; 221(2):303-308.
58. Whiteford HA, Jarvis MR, Stedman TJ, Pond S, Csernansky JG. Mianserin-induced up-regulation of serotonin receptors on normal human platelets in vivo. *Life Sci.* 1993; 53(4):371-376.
59. Takao K, Nagatani T, Kitamura Y, Kawasaki K, Hayakawa H, Yamawaki S. Chronic forced swim stress of rats increases frontal cortical 5-HT₂ receptors and the wet-dog shakes they mediate, but not frontal cortical beta-adrenoceptors. *Eur J Pharmacol.* 1995 Dec 29; 294(2-3):721-726.
60. Berton O, Aguerre S, Sarrieu A, Mormede P, Chaouloff F. Differential effects of social stress on central serotonergic activity and emotional reactivity in Lewis and spontaneously hypertensive rats. *Neuroscience.* 1998; 82(1):147-159.
61. Ossowska G, Nowak G, Klenk-Majewska B, Danilczuk Z, Zebrowska-Lupina I. Effect of imipramine on brain D-1 and 5-HT-2A receptors in a chronic unpredictable stress model in rats. *Pol J Pharmacol.* 2002 Mar-Apr; 54(2):89-93.
62. Torda T, Murgas K, Cechova E, Kiss A, Saavedra JM. Adrenergic regulation of [3H]ketanserin binding sites during immobilization stress in the rat frontal cortex. *Brain Res.* 1990; 527(2):198-203.

63. McKittrick CR, Blanchard DC, Blanchard RJ, McEwen BS, Sakai RR. Serotonin receptor binding in a colony model of chronic social stress. *Biol Psychiatry*. 1995 Mar; 37(6):383-393.
64. Fernandes C, McKittrick CR, File SE, McEwen BS. Decreased 5-HT_{1A} and increased 5-HT_{2A} receptor binding after chronic corticosterone associated with a behavioural indication of depression but not anxiety. *Psychoneuroendocrinology*. 1997 Oct; 22(7):477-491.
65. Jitsuiki H, Kagaya A, Goto S, Horiguchi J, Yamawaki S. Effect of lithium carbonate on the enhancement of serotonin 2A receptor elicited by dexamethasone. *Neuropsychobiology*. 2000; 41(2):55-61.
66. Katagiri H, Kagaya A, Nakae S, Morinobu S, Yamawaki S. Modulation of serotonin_{2A} receptor function in rats after repeated treatment with dexamethasone and L-type calcium channel antagonist nimodipine. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*. 2001 Aug; 25(6):1269-1281.
67. Kuroda Y, Mikuni M, Ogawa T, Takahashi K. Effect of ACTH, adrenalectomy and the combination treatment on the density of 5-HT₂ receptor binding sites in neocortex of rat forebrain and 5-HT₂ receptor-mediated wet-dog shake behaviors. *Psychopharmacology (Berl)*. 1992; 108(1-2):27-32.
68. Kuroda Y, Mikuni M, Nomura N, Takahashi K. Differential effect of subchronic dexamethasone treatment on serotonin-2 and beta-adrenergic receptors in the rat cerebral cortex and hippocampus. *Neurosci Lett*. 1993 Jun 11; 155(2):195-198.
69. Kozuru T, Kagaya A, Takebayashi M, Horiguchi J, Yamawaki S. Chronic electroconvulsive shock decreases (+/-) 1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane hydrochloride (DOI)-induced wet-dog shake behaviors of dexamethasone-treated rats. *Life Sciences*. 2000 Feb; 66(13):1271-1279.
70. Takao K, Nagatani T, Kitamura Y, Yamawaki S. Effects of corticosterone on 5-HT_{1A} and 5-HT₂ receptor binding and on the receptor-mediated behavioral responses of rats. *Eur J Pharmacol*. 1997 Aug 27; 333(2-3):123-128.
71. Van de Kar LD, Javed A, Zhang Y, Serres F, Raap DK, Gray TS. 5-HT_{2A} receptors stimulate ACTH, corticosterone, oxytocin, renin, and

prolactin release and activate hypothalamic CRF and oxytocin-expressing cells. *J Neurosci*. 2001 May 15; 21(10):3572-3579.

72. Zhang Y, Damjanoska KJ, Carrasco GA, Dudas B, D'Souza DN, Tetzlaff J, et al. Evidence that 5-HT_{2A} receptors in the hypothalamic paraventricular nucleus mediate neuroendocrine responses to (-)DOI. *J Neurosci*. 2002 Nov 1; 22(21):9635-9642.

73. Dinan TG. Serotonin and the regulation of hypothalamic-pituitary-adrenal axis function. *Life Sci*. 1996; 58(20):1683-1694.

74. Chaouloff F. Regulation of 5-HT receptors by corticosteroids: where do we stand? *Fundam Clin Pharmacol*. 1995; 9(3):219-233.

75. Crayton JW, Joshi I, Gulati A, Arora RC, Wolf WA. Effect of corticosterone on serotonin and catecholamine receptors and uptake sites in rat frontal cortex. *Brain Research*. 1996 Jul; 728(2):260-262.

76. Pritchett DB, Bach AW, Wozny M, Taleb O, Dal Toso R, Shih JC, et al. Structure and functional expression of cloned rat serotonin 5HT-2 receptor. *EMBO J*. 1988 Dec 20; 7(13):4135-4140.

77. Saltzman AG, Morse B, Whitman MM, Ivanshchenko Y, Jaye M, Felder S. Cloning of the human serotonin 5-HT₂ and 5-HT_{1C} receptor subtypes. *Biochem Biophys Res Commun*. 1991 Dec 31; 181(3):1469-1478.

78. Johnson MP, Loncharich RJ, Baez M, Nelson DL. Species variations in transmembrane region V of the 5-hydroxytryptamine type 2A receptor alter the structure-activity relationship of certain ergolines and tryptamines. *Mol Pharmacol*. 1994 Feb; 45(2):277-286.

79. Kao HT, Adham N, Olsen MA, Weinshank RL, Branchek TA, Hartig PR. Site-directed mutagenesis of a single residue changes the binding properties of the serotonin 5-HT₂ receptor from a human to a rat pharmacology. *FEBS Lett*. 1992 Aug 3; 307(3):324-328.

80. Stam NJ, Van Huizen F, Van Alebeek C, Brands J, Dijkema R, Tonnaer JA, et al. Genomic organization, coding sequence and functional expression of human 5-HT₂ and 5-HT_{1A} receptor genes. *Eur J Pharmacol*. 1992 Oct 1; 227(2):153-162.

81. Sparkes RS, Lan N, Klisak I, Mohandas T, Diep A, Kojis T, et al. Assignment of a serotonin 5HT-2 receptor gene (HTR2) to human

chromosome 13q14-q21 and mouse chromosome 14. *Genomics*. 1991; 9(3):461-465.

82. NCBI SNP database dbSNP Short Genetic Variation. 2012 [cited 2012 2012-11-13]; Available from: http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=3356

83. Spurlock G, Heils A, Holmans P, Williams J, D'Souza UM, Cardno A, et al. A family based association study of T102C polymorphism in 5HT2A and schizophrenia plus identification of new polymorphisms in the promoter. *Mol Psychiatry*. 1998 Jan; 3(1):42-49.

84. Parsons MJ, D'Souza UM, Arranz MJ, Kerwin RW, Makoff AJ. The -1438A/G polymorphism in the 5-hydroxytryptamine type 2A receptor gene affects promoter activity. *Biol Psychiatry*. 2004; 56(6):406-410.

85. Myers RL, Airey DC, Manier DH, Shelton RC, Sanders-Bush E. Polymorphisms in the regulatory region of the human serotonin 5-HT2A receptor gene (HTR2A) influence gene expression. *Biol Psychiatry*. 2007; 61(2):167-173.

86. Hazelwood LA, Sanders-Bush E. His452Tyr polymorphism in the human 5-HT2A receptor destabilizes the signaling conformation. *Mol Pharmacol*. 2004 Nov; 66(5):1293-1300.

87. Ozaki N, Manji H, Lubierman V, Lu SJ, Lappalainen J, Rosenthal NE, et al. A naturally occurring amino acid substitution of the human serotonin 5-HT2A receptor influences amplitude and timing of intracellular calcium mobilization. *J Neurochem*. 1997 May; 68(5):2186-2193.

88. Warren JT, Jr., Peacock ML, Rodriguez LC, Fink JK. An MspI polymorphism in the human serotonin receptor gene (HTR2): detection by DGGE and RFLP analysis. *Hum Mol Genet*. 1993 Mar; 2(3):338.

89. Kouzmenko AP, Hayes WL, Pereira AM, Dean B, Burnet PW, Harrison PJ. 5-HT2A receptor polymorphism and steady state receptor expression in schizophrenia. *Lancet*. 1997; 349(9068):1815.

90. Turecki G, Briere R, Dewar K, Antonetti T, Lesage AD, Seguin M, et al. Prediction of level of serotonin 2A receptor binding by serotonin receptor 2A genetic variation in postmortem brain samples from

subjects who did or did not commit suicide. *Am J Psychiatry*. 1999 Sep; 156(9):1456-1458.

91. Khait VD, Huang YY, Zalsman G, Oquendo MA, Brent DA, Harkavy-Friedman JM, et al. Association of serotonin 5-HT_{2A} receptor binding and the T102C polymorphism in depressed and healthy Caucasian subjects. *Neuropsychopharmacology*. 2005 Jan; 30(1):166-172.

92. Polesskaya OO, Sokolov BP. Differential expression of the "C" and "T" alleles of the 5-HT_{2A} receptor gene in the temporal cortex of normal individuals and schizophrenics. *J Neurosci Res*. 2002; 67(6):812-822.

93. Fukuda Y, Koga M, Arai M, Noguchi E, Ohtsuki T, Horiuchi Y, et al. Monoallelic and unequal allelic expression of the HTR_{2A} gene in human brain and peripheral lymphocytes. *Biol Psychiatry*. 2006; 60(12):1331-1335.

94. Pletscher A. Metabolism, transfer and storage of 5-hydroxytryptamine in blood platelets. *Br J Pharmacol Chemother*. 1968 Jan; 32(1):1-16.

95. Graf M, Pletscher A. Shape change of blood platelets--a model for cerebral 5-hydroxytryptamine receptors? *Br J Pharmacol*. 1979 Apr; 65(4):601-608.

96. Geaney DP, Schachter M, Elliot JM, Grahame-Smith DG. Characterisation of [3H]lysergic acid diethylamide binding to a 5-hydroxytryptamine receptor on human platelet membranes. *Eur J Pharmacol*. 1984 Jan 13; 97(1-2):87-93.

97. Ostrowitzki S, Rao ML, Redei J, Andres AH. Concurrence of cortex and platelet serotonin₂ receptor binding characteristics in the individual and the putative regulation by serotonin. *J Neural Transm Gen Sect*. 1993; 93(1):27-35.

98. Andres AH, Rao ML, Ostrowitzki S, Entzian W. Human brain cortex and platelet serotonin₂ receptor binding properties and their regulation by endogenous serotonin. *Life Sci*. 1993; 52(3):313-321.

99. Cook EH, Jr., Fletcher KE, Wainwright M, Marks N, Yan SY, Leventhal BL. Primary structure of the human platelet serotonin 5-HT_{2A} receptor: identify with frontal cortex serotonin 5-HT_{2A} receptor. *J Neurochem*. 1994 Aug; 63(2):465-469.

100. McBride PA, Mann JJ, McEwen B, Biegon A. Characterization of serotonin binding sites on human platelets. *Life Sci.* 1983 Nov 14; 33(20):2033-2041.
101. Elliott JM, Kent A. Comparison of [¹²⁵I]iodolysergic acid diethylamide binding in human frontal cortex and platelet tissue. *J Neurochem.* 1989 Jul; 53(1):191-196.
102. Padín JF, Rodríguez MA, Domínguez E, Dopeso-Reyes IG, Buceta M, Cano E, et al. Parallel regulation by olanzapine of the patterns of expression of 5-HT_{2A} and D₃ receptors in rat central nervous system and blood cells. *Neuropharmacology.* 2006 Sep; 51(4):923-932.
103. Pletscher A. Blood platelets as neuronal models: use and limitations. *Clin Neuropharmacol.* 1986; 9 Suppl 4:344-346.
104. Cho R, Kapur S, Du L, Hrdina P. Relationship between central and peripheral serotonin 5-HT_{2A} receptors: a positron emission tomography study in healthy individuals. *Neurosci Lett.* 1999 Feb 19; 261(3):139-142.
105. Burnet PW, Eastwood SL, Harrison PJ. Detection and quantitation of 5-HT_{1A} and 5-HT_{2A} receptor mRNAs in human hippocampus using a reverse transcriptase-polymerase chain reaction (RT-PCR) technique and their correlation with binding site densities and age. *Neurosci Lett.* 1994 Aug 29; 178(1):85-89.
106. Fowler CJ, Sjöberg E, Tiger G. Serotonin stimulation of calcium mobilisation in human platelets: choice of units of measurement, effects of age and tobacco use, and correlation with serotonin_{2A} receptor density. *Clin Chim Acta.* 1999 Sep; 287(1-2):1-18.
107. Ikemoto K, Nishimura A, Okado N, Mikuni M, Nishi K, Nagatsu I. Human midbrain dopamine neurons express serotonin 2A receptor: an immunohistochemical demonstration. *Brain Res.* 2000 Jan 24; 853(2):377-380.
108. McKeith IG, Marshall EF, Ferrier IN, Armstrong MM, Kennedy WN, Perry RH, et al. 5-HT receptor binding in post-mortem brain from patients with affective disorder. *J Affect Disord.* 1987 1987 Jul-Aug; 13(1):67-74.

109. Yates M, Leake A, Candy JM, Fairbairn AF, McKeith IG, Ferrier IN. 5HT₂ receptor changes in major depression. *Biol Psychiatry*. 1990 Mar; 27(5):489-496.
110. Arora RC, Meltzer HY. Increased serotonin₂ (5-HT₂) receptor binding as measured by 3H-lysergic acid diethylamide (3H-LSD) in the blood platelets of depressed patients. *Life Sci*. 1989; 44(11):725-734.
111. Biegon A, Weizman A, Karp L, Ram A, Tiano S, Wolff M. Serotonin 5-HT₂ receptor binding on blood platelets--a peripheral marker for depression? *Life Sci*. 1987 Nov 30; 41(22):2485-2492.
112. Pandey GN, Pandey SC, Janicak PG, Marks RC, Davis JM. Platelet serotonin-2 receptor binding sites in depression and suicide. *Biol Psychiatry*. 1990; 28(3):215-222.
113. Hrdina PD, Bakish D, Chudzik J, Ravindran A, Lapierre YD. Serotonergic markers in platelets of patients with major depression: upregulation of 5-HT₂ receptors. *J Psychiatry Neurosci*. 1995 Jan; 20(1):11-19.
114. Arora RC, Meltzer HY. Serotonergic measures in the brains of suicide victims: 5-HT₂ binding sites in the frontal cortex of suicide victims and control subjects. *Am J Psychiatry*. 1989 Jun; 146(6):730-736.
115. Arango V, Ernsberger P, Marzuk PM, Chen JS, Tierney H, Stanley M, et al. Autoradiographic demonstration of increased serotonin 5-HT₂ and beta-adrenergic receptor binding sites in the brain of suicide victims. *Arch Gen Psychiatry*. 1990 Nov; 47(11):1038-1047.
116. Mann JJ, Stanley M, McBride PA, McEwen BS. Increased serotonin₂ and beta-adrenergic receptor binding in the frontal cortices of suicide victims. *Arch Gen Psychiatry*. 1986 Oct; 43(10):954-959.
117. Stanley M, Mann JJ. Increased serotonin-2 binding sites in frontal cortex of suicide victims. *Lancet*. 1983 Jan; 1(8318):214-216.
118. Pandey GN, Pandey SC, Dwivedi Y, Sharma RP, Janicak PG, Davis JM. Platelet serotonin-2A receptors: a potential biological marker for suicidal behavior. *Am J Psychiatry*. 1995 Jun; 152(6):850-855.
119. McBride PA, Brown RP, DeMeo M, Keilp J, Mieczkowski T, Mann JJ. The relationship of platelet 5-HT₂ receptor indices to major

depressive disorder, personality traits, and suicidal behavior. *Biol Psychiatry*. 1994 Mar 1; 35(5):295-308.

120. Underwood MD, Kassir SA, Bakalian MJ, Galfalvy H, Mann JJ, Arango V. Neuron density and serotonin receptor binding in prefrontal cortex in suicide. *Int J Neuropsychopharmacol*. 2012; 15(4):435-447.

121. Norton N, Owen MJ. HTR2A: association and expression studies in neuropsychiatric genetics. *Ann Med*. 2005; 37(2):121-129.

122. Serretti A, Drago A, De Ronchi D. HTR2A gene variants and psychiatric disorders: a review of current literature and selection of SNPs for future studies. *Curr Med Chem*. 2007; 14(19):2053-2069.

123. Li D, Duan Y, He L. Association study of serotonin 2A receptor (5-HT_{2A}) gene with schizophrenia and suicidal behavior using systematic meta-analysis. *Biochem Biophys Res Commun*. 2006; 340(3):1006-1015.

124. Arora RC, Meltzer HY. Serotonin₂ receptor binding in blood platelets of schizophrenic patients. *Psychiatry Res*. 1993; 47(2):111-119.

125. Arranz B, Rosel P, Sarro S, Ramirez N, Duenas R, Cano R, et al. Altered platelet serotonin 5-HT_{2A} receptor density but not second messenger inositol trisphosphate levels in drug-free schizophrenic patients. *Psychiatry Res*. 2003; 118(2):165-174.

126. Govitrapong P, Chagkutip J, Turakitwanakan W, Srikiatkachorn A. Platelet 5-HT_{2A} receptors in schizophrenic patients with and without neuroleptic treatment. *Psychiatry Res*. Ireland; 2000:41-50.

127. Pandey SC, Sharma RP, Janicak PG, Marks RC, Davis JM, Pandey GN. Platelet serotonin-2 receptors in schizophrenia: effects of illness and neuroleptic treatment. *Psychiatry Res*. 1993 Jul; 48(1):57-68.

128. Erritzoe D, Rasmussen H, Kristiansen KT, Frokjaer VG, Haugbol S, Pinborg L, et al. Cortical and subcortical 5-HT_{2A} receptor binding in neuroleptic-naive first-episode schizophrenic patients. *Neuropsychopharmacology*. 2008; 33(10):2435-2441.

129. Verhoeff NP, Meyer JH, Kecojevic A, Hussey D, Lewis R, Tauscher J, et al. A voxel-by-voxel analysis of [18F]setoperone PET data

shows no substantial serotonin 5-HT(2A) receptor changes in schizophrenia. *Psychiatry Res.* 2000; 99(3):123-135.

130. Ngan ET, Yatham LN, Ruth TJ, Liddle PF. Decreased serotonin 2A receptor densities in neuroleptic-naive patients with schizophrenia: A PET study using [(18)F]setoperone. *Am J Psychiatry.* 2000 Jun; 157(6):1016-1018.

131. Trichard C, Paillere-Martinot ML, Attar-Levy D, Blin J, Feline A, Martinot JL. No serotonin 5-HT2A receptor density abnormality in the cortex of schizophrenic patients studied with PET. *Schizophr Res.* 1998; 31(1):13-17.

132. Williams J, McGuffin P, Nöthen M, Owen MJ. Meta-analysis of association between the 5-HT2a receptor T102C polymorphism and schizophrenia. EMASS Collaborative Group. European Multicentre Association Study of Schizophrenia. *Lancet.* 1997 Apr; 349(9060):1221.

133. Abdolmaleky HM, Faraone SV, Glatt SJ, Tsuang MT. Meta-analysis of association between the T102C polymorphism of the 5HT2a receptor gene and schizophrenia. *Schizophrenia Research.* 2004 Mar; 67(1):53-62.

134. Neovius M, Simard JF, Askling J. Nationwide prevalence of rheumatoid arthritis and penetration of disease-modifying drugs in Sweden. *Ann Rheum Dis.* 2011 Apr; 70(4):624-629.

135. Rantapää-Dahlqvist S, Jacobsson L. Reumatoid artrit/ledgångsreumatism. In: Klareskog L, Saxne T, Enman Y, eds. *Reumatologi.* 2., [rev.] uppl. ed. Stockholm: Studentlitteratur; 2011:423 s.

136. 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 and Rheumatism.* 1988 Mar; 31(3):315-324.

137. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO, 3rd, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum.* 2010 Sep; 62(9):2569-2581.

138. Eyre S, Bowes J, Diogo D, Lee A, Barton A, Martin P, et al. High-density genetic mapping identifies new susceptibility loci for rheumatoid arthritis. *Nat Genet.* 2012 Nov 11; 44(12):1336-1340.

139. Karlson EW, Chang SC, Cui J, Chibnik LB, Fraser PA, De Vivo I, et al. Gene-environment interaction between HLA-DRB1 shared epitope and heavy cigarette smoking in predicting incident rheumatoid arthritis. *Ann Rheum Dis*. 2010 Jan; 69(1):54-60.
140. Baecklund E, Forsblad d'Elia H, Turesson C. Guidelines for the Pharmaceutical Management of Rheumatoid Arthritis Swedish Society of Rheumatology, April 14, 2011. 2011 [cited 2011 December 14]; Available from: http://www.svenskreumatologi.se/kunder/srf/sites/default/files/49/Guidelines_for_the_Pharmaceutical_Management_of_Rheumatoid_Arthritis.pdf
141. Smolen JS, Landewe R, Breedveld FC, Dougados M, Emery P, Gaujoux-Viala C, et al. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs. *Ann Rheum Dis*. 2010 Jun; 69(6):964-975.
142. Boers M. The case for corticosteroids in the treatment of early rheumatoid arthritis. *Rheumatology (Oxford)*. 1999 Feb; 38(2):95-97.
143. Morrison E, Capell HA. Corticosteroids in rheumatoid arthritis--the case against. *Rheumatology (Oxford)*. 1999 Feb; 38(2):97-100.
144. Anderson JK, Zimmerman L, Caplan L, Michaud K. Measures of rheumatoid arthritis disease activity: Patient (PtGA) and Provider. *Arthritis Care Res (Hoboken)*. 2011 Nov; 63 Suppl 11:S14-36.
145. Jacobsson LT, Hetland ML. New remission criteria for RA: 'modern times' in rheumatology--not a silent film, rather a 3D movie. *Ann Rheum Dis*. 2011; 70(3):401-403.
146. Chen SJ, Chao YL, Chen CY, Chang CM, Wu EC, Wu CS, et al. Prevalence of autoimmune diseases in in-patients with schizophrenia: nationwide population-based study. *Br J Psychiatry*. 2012 May; 200(5):374-380.
147. Eaton WW, Hayward C, Ram R. Schizophrenia and rheumatoid-arthritis - a review. *Schizophrenia Research*. 1992 Mar; 6(3):181-192.
148. Osterberg E. Schizophrenia and rheumatic disease. A study on the concurrence of inflammatory joint diseases and a review of 58 case-records. *Acta Psychiatr Scand*. 1978 Oct; 58(4):339-359.

149. Allebeck P, Rodvall Y, Wistedt B. Incidence of rheumatoid arthritis among patients with schizophrenia, affective psychosis and neurosis. *Acta Psychiatr Scand*. 1985 Jun; 71(6):615-619.
150. Goldacre M, Kurina L, Yeates D, Seagroatt V, Gill L. Use of large medical databases to study associations between diseases. *Qjm*. 2000 Oct; 93(10):669-675.
151. Malek-Ahmadi P. Rheumatoid arthritis and schizophrenia: are they mutually exclusive? *Semin Arthritis Rheum*. 1985 Aug; 15(1):70-72.
152. Mors O, Mortensen PB, Ewald H. A population-based register study of the association between schizophrenia and rheumatoid arthritis. *Schizophr Res*. 1999 Nov 9; 40(1):67-74.
153. Spector TD, Silman AJ. Rheumatoid arthritis, diabetes, and schizophrenia. *Lancet*. 1990 Jan 27; 335(8683):228-229.
154. Taylor WM. Schizophrenia, rheumatoid arthritis and tryptophan metabolism. *J Clin Psychiatry*. 1978 Jun; 39(6):499-503.
155. Crunkhorn P, Meacock SC. Mediators of the inflammation induced in the rat paw by carrageenin. *Br J Pharmacol*. 1971 Jul; 42(3):392-402.
156. Maling HM, Webster ME, Williams MA, Saul W, Anderson W, Jr. Inflammation induced by histamine, serotonin, bradykinin and compound 48-80 in the rat: antagonists and mechanisms of action. *J Pharmacol Exp Ther*. 1974 Nov; 191(2):300-310.
157. Harbuz MS, PerveenGill Z, Lalies MD, Jessop DS, Lightman SL, Chowdrey HS. The role of endogenous serotonin in adjuvant-induced arthritis in the rat. *British Journal of Rheumatology*. 1996 Feb; 35(2):112-116.
158. Sufka KJ, Schomburg FM, Giordano J. Receptor mediation of 5-HT-induced inflammation and nociception in rats. *Pharmacol Biochem Behav*. 1992 Jan; 41(1):53-56.
159. Pierce PA, Xie GX, Peroutka SJ, Green PG, Levine JD. 5-hydroxytryptamine-induced synovial plasma extravasation is mediated via 5-hydroxytryptamine_{2a} receptors on sympathetic efferent terminals. *Journal of Pharmacology and Experimental Therapeutics*. 1995 Oct; 275(1):502-508.

160. Pertsch M, Krause E, Hirschelmann R. A comparison of serotonin (5-HT) blood levels and activity of 5-HT₂ antagonists in adjuvant arthritic Lewis and Wistar rats. *Agents Actions*. 1993; 38 Spec No:C98-101.
161. Baharav E, Bar M, Taler M, Gil-Ad I, Karp L, Weinberger A, et al. Immunomodulatory effect of sertraline in a rat model of rheumatoid arthritis. *Neuroimmunomodulation*. 2012; 19(5):309-318.
162. Sacre S, Medghalchi M, Gregory B, Brennan F, Williams R. Fluoxetine and citalopram exhibit potent antiinflammatory activity in human and murine models of rheumatoid arthritis and inhibit toll-like receptors. *Arthritis Rheum*. 2010 Mar; 62(3):683-693.
163. Crawford N. Some observations on the blood serotonin levels in rheumatoid arthritis with a study of platelet serotonin absorption. *Clin Chim Acta*. 1969 Jan; 23(1):139-146.
164. Genefke IK, Garel A, Mandel P. Factors influencing free serotonin in human plasma. *Clin Chim Acta*. 1968 Apr; 20(1):61-67.
165. Cunningham TJ, Medcalf RL, Mathews JD, Muirden KD. Platelet releasing activity in sera of patients with rheumatoid vasculitis. *Ann Rheum Dis*. 1986 Jan; 45(1):15-20.
166. Little CH, Stewart AG, Fennessy MR. Platelet serotonin release in rheumatoid arthritis: a study in food-intolerant patients. *Lancet*. 1983 Aug 6; 2(8345):297-299.
167. Kopp S, Alstergren P. Blood serotonin and joint pain in seropositive versus seronegative rheumatoid arthritis. *Mediators Inflamm*. 2002 Aug; 11(4):211-217.
168. Garelli R. [Action of a serotonin antagonist on rheumatic inflammation]. *Reumatismo*. 1960 Mar-Apr; 12:92-98.
169. Salvarani C, Cantini F, Boiardi L, Hunder GG. Polymyalgia rheumatica and giant-cell arteritis. *N Engl J Med*. 2002 Jul; 347(4):261-271.
170. Nordborg E, Nordborg C, Uddhammar A. Temporalisarterit och polymyalgia reumatika. In: Klareskog L, Saxne T, Enman Y, eds. *Reumatologi*. Lund: Studentlitteratur; 2011.
171. Edwards IR, Aronson JK. Adverse drug reactions: definitions, diagnosis, and management. *Lancet*. 2000 Oct 7; 356(9237):1255-1259.

172. O'Donnell JM SR. Drug Therapy of Depression and Anxiety Disorders. *Goodman & Gilman's The Pharmacological Basis of Therapeutics* 12th ed. 2011(Chapter 15.).
173. Choi S. Nefazodone (Serzone) withdrawn because of hepatotoxicity. *CMAJ*. 2003 Nov 25; 169(11):1187.
174. Stolt P, Bengtsson C, Nordmark B, Lindblad S, Lundberg I, Klareskog L, et al. Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases. *Ann Rheum Dis*. 2003 Sep; 62(9):835-841.
175. Padyukov L, Silva C, Stolt P, Alfredsson L, Klareskog L. A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive rheumatoid arthritis. *Arthritis Rheum*. 2004 Oct; 50(10):3085-3092.
176. Spigset O, Mjörndal T. Serotonin 5-HT_{2A} receptor binding in platelets from healthy subjects as studied by [3H]-lysergic acid diethylamide (3H-LSD): intra- and interindividual variability. *Neuropsychopharmacology*. 1997 Apr; 16(4):285-293.
177. Steckler T, Ruggeberg-Schmidt K, Muller-Oerlinghausen B. Human platelet 5-HT₂ receptor binding sites re-evaluated: a criticism of current techniques [corrected]. *J Neural Transm Gen Sect*. 1993; 92(1):11-24.
178. Hartig PR, Kadan MJ, Evans MJ, Krohn AM. 125I-LSD: a high sensitivity ligand for serotonin receptors. *Eur J Pharmacol*. 1983 May 6; 89(3-4):321-322.
179. Backstrom JR, Chang MS, Chu H, Niswender CM, Sanders-Bush E. Agonist-directed signaling of serotonin 5-HT_{2C} receptors: differences between serotonin and lysergic acid diethylamide (LSD). *Neuropsychopharmacology*. 1999; 21(2 Suppl):77S-81S.
180. Knight AR, Misra A, Quirk K, Benwell K, Revell D, Kennett G, et al. Pharmacological characterisation of the agonist radioligand binding site of 5-HT(2A), 5-HT(2B) and 5-HT(2C) receptors. *Naunyn Schmiedebergs Arch Pharmacol*. 2004 Aug; 370(2):114-123.
181. Bengtsson BO, Wiholm BE, Myrhed M, Walinder J. Adverse experiences during treatment with zimeldine on special licence in Sweden. *Int Clin Psychopharmacol*. 1994 Spring; 9(1):55-61.

182. Nilsson BS. Adverse reactions in connection with zimeldine treatment--a review. *Acta Psychiatr Scand Suppl.* 1983; 308:115-119.
183. Langlois R, Cournoyer G, de Montigny C, Caillé G. High incidence of multisystemic reactions to zimeldine. *Eur J Clin Pharmacol.* 1985; 28(1):67-71.
184. WHO. DDD alterations from 1982-2011. ATC/DDD alterations, cumulative lists. 2012 2011-09-16. [cited 2012 2012-11-21.]; Available from: http://www.whooc.no/atc_ddd_alterations_cumulative_/ddd_alterations/.
185. Corash L, Costa JL, Shafer B, Donlon JA, Murphy D. Heterogeneity of human whole blood platelet subpopulations. III. Density-dependent differences in subcellular constituents. *Blood.* 1984 Jul; 64(1):185-193.
186. Hensler JG, Truett KA. Effect of chronic serotonin-2 receptor agonist or antagonist administration on serotonin-1A receptor sensitivity. *Neuropsychopharmacology.* 1998 Nov; 19(5):354-364.
187. Masse F, Hascoet M, Dailly E, Bourin M. Effect of noradrenergic system on the anxiolytic-like effect of DOI (5-HT_{2A/2C} agonists) in the four-plate test. *Psychopharmacology (Berl).* 2006 Jan; 183(4):471-481.
188. Matsumoto K, Mizowaki M, Thongpraditchote S, Murakami Y, Watanabe H. alpha₂-Adrenoceptor antagonists reverse the 5-HT₂ receptor antagonist suppression of head-twitch behavior in mice. *Pharmacol Biochem Behav.* 1997; 56(3):417-422.
189. de Chaffoy de Courcelles D, Roevens P, Van Belle H, De Clerck F. The synergistic effect of serotonin and epinephrine on the human platelet at the level of signal transduction. *FEBS Lett.* 1987; 219(2):283-288.
190. Schmidt CJ, Sorensen SM, Kehne JH, Carr AA, Palfreyman MG. The role of 5-HT_{2A} receptors in antipsychotic activity. *Life Sci.* 1995; 56(25):2209-2222.
191. Van Oekelen D, Megens A, Meert T, Luyten WH, Leysen JE. Functional study of rat 5-HT_{2A} receptors using antisense oligonucleotides. *J Neurochem.* 2003; 85(5):1087-1100.

192. Erritzoe D, Holst K, Frokjaer VG, Licht CL, Kalbitzer J, Nielsen FA, et al. A nonlinear relationship between cerebral serotonin transporter and 5-HT(2A) receptor binding: an in vivo molecular imaging study in humans. *J Neurosci*. 2010 Mar 3; 30(9):3391-3397.
193. Toth M, Shenk T. Antagonist-mediated down-regulation of 5-hydroxytryptamine type 2 receptor gene expression: modulation of transcription. *Mol Pharmacol*. 1994 Jun; 45(6):1095-1100.
194. Trajkovska V, Kirkegaard L, Krey G, Marcussen AB, Thomsen MS, Chourbaji S, et al. Activation of glucocorticoid receptors increases 5-HT2A receptor levels. *Exp Neurol*. 2009; 218(1):83-91.
195. Islam A, Thompson KS, Akhtar S, Handley SL. Increased 5-HT2A receptor expression and function following central glucocorticoid receptor knockdown in vivo. *Eur J Pharmacol*. 2004 Oct 19; 502(3):213-220.
196. Garlow SJ, Ciaranello RD. Transcriptional control of the rat serotonin-2 receptor gene. *Brain Res Mol Brain Res*. 1995 Jul; 31(1-2):201-209.
197. Dickens C, McGowan L, Clark-Carter D, Creed F. Depression in rheumatoid arthritis: a systematic review of the literature with meta-analysis. *Psychosom Med*. 2002 Jan-Feb; 64(1):52-60.
198. Moll LT, Gormsen L, Pfeiffer-Jensen M. [Higher prevalence of depression in patients with rheumatoid arthritis--a systematic review]. *Ugeskr Laeger*. 2011 Oct 10; 173(41):2564-2568.
199. Pincus T, Griffith J, Pearce S, Isenberg D. Prevalence of self-reported depression in patients with rheumatoid arthritis. *Br J Rheumatol*. 1996 Sep; 35(9):879-883.
200. Sato E, Nishimura K, Nakajima A, Okamoto H, Shinozaki M, Inoue E, et al. Major depressive disorder in patients with rheumatoid arthritis. *Mod Rheumatol*. 2012 May 23.
201. Coury F, Rossat A, Tebib A, Letroublon MC, Gagnard A, Fantino B, et al. Rheumatoid arthritis and fibromyalgia: a frequent unrelated association complicating disease management. *J Rheumatol*. 2009 Jan; 36(1):58-62.
202. Kapoor SR, Hider SL, Brownfield A, Matthey DL, Packham JC. Fibromyalgia in patients with rheumatoid arthritis: driven by depression

or joint damage? Clin Exp Rheumatol. 2011 Nov-Dec; 29(6 Suppl 69):S88-91.

203. Bondy B, Spaeth M, Offenbaecher M, Glatzeder K, Stratz T, Schwarz M, et al. The T102C polymorphism of the 5-HT_{2A}-receptor gene in fibromyalgia. Neurobiology of Disease. 1999 Oct; 6(5):433-439.

204. Gürsoy S, Erdal E, Herken H, Madenci E, Alaşehirli B. Association of T102C polymorphism of the 5-HT_{2A} receptor gene with psychiatric status in fibromyalgia syndrome. Rheumatol Int. 2001 Oct; 21(2):58-61.

205. Tander B, Gunes S, Boke O, Alayli G, Kara N, Bagci H, et al. Polymorphisms of the serotonin-2A receptor and catechol-O-methyltransferase genes: a study on fibromyalgia susceptibility. Rheumatol Int. 2008 May; 28(7):685-691.

206. Lee YH, Choi SJ, Ji JD, Song GG. Candidate gene studies of fibromyalgia: a systematic review and meta-analysis. Rheumatol Int. 2012 Feb; 32(2):417-426.

207. Nicholl BI, Holliday KL, Macfarlane GJ, Thomson W, Davies KA, O'Neill TW, et al. Association of HTR_{2A} polymorphisms with chronic widespread pain and the extent of musculoskeletal pain: results from two population-based cohorts. Arthritis Rheum. 2011 Mar; 63(3):810-818.

208. Kaumann AJ, Levy FO. 5-hydroxytryptamine receptors in the human cardiovascular system. Pharmacol Ther. 2006 Sep; 111(3):674-706.

209. Vikenes K, Farstad M, Nordrehaug JE. Serotonin is associated with coronary artery disease and cardiac events. Circulation. 1999 Aug 3; 100(5):483-489.

210. Nilsson T, Longmore J, Shaw D, Pantev E, Bard JA, Branchek T, et al. Characterisation of 5-HT receptors in human coronary arteries by molecular and pharmacological techniques. Eur J Pharmacol. 1999; 372(1):49-56.

211. De Clerck F, Van Nueten JM. Platelet-mediated vascular contractions: inhibition of the serotonergic component by ketanserin. Thromb Res. 1982; 27(6):713-727.

212. Avina-Zubieta JA, Choi HK, Sadatsafavi M, Etminan M, Esdaile JM, Lacaille D. Risk of cardiovascular mortality in patients with

rheumatoid arthritis: a meta-analysis of observational studies. *Arthritis Rheum.* 2008 Dec 15; 59(12):1690-1697.

213. Yamada S, Akita H, Kanazawa K, Ishida T, Hirata K, Ito K, et al. T102C polymorphism of the serotonin (5-HT) 2A receptor gene in patients with non-fatal acute myocardial infarction. *Atherosclerosis.* 2000; 150(1):143-148.

214. Coto E, Reguero JR, Alvarez V, Morales B, Batalla A, Gonzalez P, et al. 5-Hydroxytryptamine 5-HT_{2A} receptor and 5-hydroxytryptamine transporter polymorphisms in acute myocardial infarction. *Clin Sci (Lond).* 2003; 104(3):241-245.

215. Markovitz JH, Tolbert L, Winders SE. Increased serotonin receptor density and platelet GPIIb/IIIa activation among smokers. *Arterioscler Thromb Vasc Biol.* 1999 Mar; 19(3):762-766.

216. Polina ER, Contini V, Hutz MH, Bau CH. The serotonin 2A receptor gene in alcohol dependence and tobacco smoking. *Drug Alcohol Depend.* 2009; 101(1-2):128-131.

217. White MJ, Young RM, Morris CP, Lawford BR. Cigarette smoking in young adults: the influence of the HTR2A T102C polymorphism and punishment sensitivity. *Drug Alcohol Depend.* Ireland: Crown 2010. Published by Elsevier Ireland Ltd; 2011:140-146.

218. do Prado-Lima PA, Chatkin JM, Taufer M, Oliveira G, Silveira E, Neto CA, et al. Polymorphism of 5HT_{2A} serotonin receptor gene is implicated in smoking addiction. *Am J Med Genet B Neuropsychiatr Genet.* 2004 Jul 1; 128B(1):90-93.

219. Huang S, Cook DG, Hinks LJ, Chen XH, Ye S, Gilg JA, et al. CYP2A6, MAOA, DBH, DRD4, and 5HT_{2A} genotypes, smoking behaviour and cotinine levels in 1518 UK adolescents. *Pharmacogenet Genomics.* 2005; 15(12):839-850.

220. Terayama H, Itoh M, Fukunishi I, Iwahashi K. The serotonin-2A receptor polymorphism and smoking behavior in Japan. *Psychiatr Genet.* 2004 Dec; 14(4):195-197.

221. Harbuz MS, Marti O, Lightman SL, Jessop DS. Alteration of central serotonin modifies onset and severity of adjuvant-induced arthritis in the rat. *British Journal of Rheumatology.* 1998 Oct; 37(10):1077-1083.

222. Harbuz MS, Conde GL, Marti O, Lightman SL, Jessop DS. The hypothalamic-pituitary-adrenal axis in autoimmunity. *Ann N Y Acad Sci.* 1997 Aug 14; 823:214-224.
223. Grubb BD, McQueen DS, Iggo A, Birrell GJ, Dutia MB. A study of 5-HT-receptors associated with afferent nerves located in normal and inflamed rat ankle joints. *Agents Actions.* 1988 Dec; 25(3-4):216-218.
224. Zhang YQ, Gao X, Ji GC, Wu GC. Expression of 5-HT_{2A} receptor mRNA in rat spinal dorsal horn and some nuclei of brainstem after peripheral inflammation. *Brain Res.* 2001 May 4; 900(1):146-151.
225. Kjorsvik Bertelsen A, Warsame Afrah A, Gustafsson H, Tjolsen A, Hole K, Stiller CO. Stimulation of spinal 5-HT_{2A/2C} receptors potentiates the capsaicin-induced in vivo release of substance P-like immunoreactivity in the rat dorsal horn. *Brain Res.* 2003 Oct 10; 987(1):10-16.
226. Menkes CJ, Renoux M, Laoussadi S, Mauborgne A, Bruxelles J, Cesselin F. Substance P levels in the synovium and synovial fluid from patients with rheumatoid arthritis and osteoarthritis. *J Rheumatol.* 1993 Apr; 20(4):714-717.
227. Lotz M, Carson DA, Vaughan JH. Substance P activation of rheumatoid synoviocytes: neural pathway in pathogenesis of arthritis. *Science.* 1987 Feb 20; 235(4791):893-895.
228. Marabini S, Matucci-Cerinic M, Geppetti P, Del Bianco E, Marchesoni A, Tosi S, et al. Substance P and somatostatin levels in rheumatoid arthritis, osteoarthritis, and psoriatic arthritis synovial fluid. *Ann N Y Acad Sci.* 1991; 632:435-436.
229. Grimsholm O, Rantapaa-Dahlqvist S, Forsgren S. Levels of gastrin-releasing peptide and substance P in synovial fluid and serum correlate with levels of cytokines in rheumatoid arthritis. *Arthritis Res Ther.* 2005; 7(3):R416-426.
230. Matucci-Cerinic M, Partsch G, Marabini S, Cagnoni M. High levels of substance P in rheumatoid arthritis synovial fluid. Lack of substance P production by synoviocytes in vitro. *Clin Exp Rheumatol.* 1991 Jul-Aug; 9(4):440-441.
231. Colli S, Maderna P, Tremoli E, Colombo F, Canesi B. Platelet function in rheumatoid arthritis. *Scand J Rheumatol.* 1982; 11(3):139-143.

232. Shapleigh C, Valone FH, Schur PH, Goetzl EJ, Austen KF. Platelet-activating activity in synovial fluids of patients with rheumatoid arthritis, juvenile rheumatoid arthritis, gout, and noninflammatory arthropathies. *Arthritis Rheum.* 1980 Jul; 23(7):800-807.
233. Weissbarth E, Baruth B, Mielke H, Liman W, Deicher H. Platelets as target cells in rheumatoid arthritis and systemic lupus erythematosus: a platelet specific immunoglobulin inducing the release reaction. *Rheumatol Int.* 1982; 2(2):67-73.
234. Boilard E, Blanco P, Nigrovic PA. Platelets: active players in the pathogenesis of arthritis and SLE. *Nat Rev Rheumatol.* 2012; 8(9):534-542.
235. Cloutier N, Pare A, Farndale RW, Schumacher HR, Nigrovic PA, Lacroix S, et al. Platelets can enhance vascular permeability. *Blood.* 2012; 120(6):1334-1343.
236. Henning U, Krieger K, Klimke A. Specific binding of 3H-spiperone to peripheral blood cells: relevance for the interpretation of binding studies in psychiatric disorders. *Prog Neuropsychopharmacol Biol Psychiatry.* 1999 Feb; 23(2):225-241.
237. Lonne-Rahm SB, Rickberg H, El-Nour H, Marin P, Azmitia EC, Nordlind K. Neuroimmune mechanisms in patients with atopic dermatitis during chronic stress. *J Eur Acad Dermatol Venereol.* 2008 Jan; 22(1):11-18.
238. Inoue M, Okazaki T, Kitazono T, Mizushima M, Omata M, Ozaki S. Regulation of antigen-specific CTL and Th1 cell activation through 5-Hydroxytryptamine 2A receptor. *Int Immunopharmacol.* 2011 Jan; 11(1):67-73.
239. Young MR, Kut JL, Coogan MP, Wright MA, Young ME, Matthews J. Stimulation of splenic T-lymphocyte function by endogenous serotonin and by low-dose exogenous serotonin. *Immunology.* 1993 Nov; 80(3):395-400.
240. Ameisen JC, Meade R, Askenase PW. A new interpretation of the involvement of serotonin in delayed-type hypersensitivity. Serotonin-2 receptor antagonists inhibit contact sensitivity by an effect on T cells. *J Immunol.* 1989 May 1; 142(9):3171-3179.

241. Nordlind K, Sundstrom E, Bondesson L. Inhibiting effects of serotonin antagonists on the proliferation of mercuric chloride stimulated human peripheral blood T lymphocytes. *Int Arch Allergy Immunol.* 1992; 97(2):105-108.
242. Seidel MF, Fiebich BL, Ulrich-Merzenich G, Candelario-Jalil E, Koch FW, Vetter H. Serotonin mediates PGE2 overexpression through 5-HT2A and 5-HT3 receptor subtypes. *Rheumatol Int.* 2008 Aug; 28(10):1017-1022.
243. Arzt E, Costas M, Finkielman S, Nahmod VE. Serotonin inhibition of tumor necrosis factor- α synthesis by human monocytes. *Life Sci.* 1991; 48(26):2557-2562.
244. Yu B, Becnel J, Zerfaoui M, Rohatgi R, Boulares AH, Nichols CD. Serotonin 5-hydroxytryptamine(2A) receptor activation suppresses tumor necrosis factor- α -induced inflammation with extraordinary potency. *J Pharmacol Exp Ther.* 2008 Nov; 327(2):316-323.
245. Ullmer C, Schmuck K, Kalkman HO, Lubbert H. Expression of serotonin receptor mRNAs in blood vessels. *FEBS Lett.* 1995 Aug 21; 370(3):215-221.
246. Seddighzadeh M, Korotkova M, Kallberg H, Ding B, Daha N, Kurreeman FA, et al. Evidence for interaction between 5-hydroxytryptamine (serotonin) receptor 2A and MHC type II molecules in the development of rheumatoid arthritis. *Eur J Hum Genet.* 2010 Jul; 18(7):821-826.
247. Hood SD, Argyropoulos SV, Nutt DJ. Arthritis and serotonergic antidepressants. *J Clin Psychopharmacol.* 2001 Aug; 21(4):458-461.
248. Passier A, van Puijenbroek E. Mirtazapine-induced arthralgia. *Br J Clin Pharmacol.* 2005; 60(5):570-572.
249. Jolliet P, Veyrac G, Bourin M. First report of mirtazapine-induced arthralgia. *Eur Psychiatry.* 2001; 16(8):503-505.
250. Ostensen M, Myhr K. Mianserin as a cause of arthritis. *Br J Rheumatol.* 1991 Feb; 30(1):74-75.
251. Kraus T, Haack M, Schuld A, Hinze-Selch D, Koethe D, Pollmacher T. Body weight, the tumor necrosis factor system, and leptin

production during treatment with mirtazapine or venlafaxine. *Pharmacopsychiatry*. 2002 Nov; 35(6):220-225.

252. Barker EL, Blakely RD. Identification of a single amino acid, phenylalanine 586, that is responsible for high affinity interactions of tricyclic antidepressants with the human serotonin transporter. *Mol Pharmacol*. 1996 Oct; 50(4):957-965.

253. Friese MA, Montalban X, Willcox N, Bell JI, Martin R, Fugger L. The value of animal models for drug development in multiple sclerosis. *Brain*. 2006 Aug; 129(Pt 8):1940-1952.

254. Roep BO, Atkinson M, von Herrath M. Satisfaction (not) guaranteed: re-evaluating the use of animal models of type 1 diabetes. *Nat Rev Immunol*. 2004 Dec; 4(12):989-997.

255. Pazos A, Hoyer D, Palacios JM. Mesulergine, a selective serotonin-2 ligand in the rat cortex, does not label these receptors in porcine and human cortex: evidence for species differences in brain serotonin-2 receptors. *Eur J Pharmacol*. 1984 Nov 27; 106(3):531-538.

256. Sparrow EM, Wilhelm DL. Species differences in susceptibility to capillary permeability factors: histamine, 5-hydroxytryptamine and compound 48/80. *J Physiol*. 1957 Jun 18; 137(1):51-65.

257. Heistad DD, Baumbach GL, Faraci FM, Armstrong ML. Sick vessel syndrome: vascular changes in hypertension and atherosclerosis. *J Hum Hypertens*. 1995 Jun; 9(6):449-453.

258. Heistad DD, Armstrong ML, Baumbach GL, Faraci FM. Sick vessel syndrome. Recovery of atherosclerotic and hypertensive vessels. *Hypertension*. 1995 Sep; 26(3):509-513.

259. Buckland PR, D'Souza U, Maher NA, McGuffin P. The effects of antipsychotic drugs on the mRNA levels of serotonin 5HT2A and 5HT2C receptors. *Brain Res Mol Brain Res*. 1997 Aug; 48(1):45-52.

260. Kubera M, Maes M, Kenis G, Kim YK, Lason W. Effects of serotonin and serotonergic agonists and antagonists on the production of tumor necrosis factor alpha and interleukin-6. *Psychiatry Res*. 2005 Apr 30; 134(3):251-258.

261. Mutlu N, Erdal ME, Herken H, Oz G, Bayazit YA. T102C polymorphism of the 5-HT2A receptor gene may be associated with temporomandibular dysfunction. *Oral Dis.* 2004 Nov; 10(6):349-352.
262. Bixo M, Allard P, Bäckström T, Mjörndal T, Nyberg S, Spigset O, et al. Binding of [3H]paroxetine to serotonin uptake sites and of [3H]lysergic acid diethylamide to 5-HT2A receptors in platelets from women with premenstrual dysphoric disorder during gonadotropin releasing hormone treatment. *Psychoneuroendocrinology.* 2001 Aug; 26(6):551-564.
263. Spigset O, Andersen T, Hägg S, Mjörndal T. Enhanced platelet serotonin 5-HT2A receptor binding in anorexia nervosa and bulimia nervosa. *Eur Neuropsychopharmacol.* 1999 Dec; 9(6):469-473.
264. Peroutka SJ. Serotonin receptor variants in disease: new therapeutic opportunities? *Ann N Y Acad Sci.* 1998 Dec; 861:16-25.
265. Humphreys K, Grankvist A, Leu M, Hall P, Liu J, Ripatti S, et al. The genetic structure of the Swedish population. *PLoS One.* 2011; 6(8):e22547.
266. Arranz M, Collier D, Sodhi M, Ball D, Roberts G, Price J, et al. Association between clozapine response and allelic variation in 5-HT2A receptor gene. *Lancet.* 1995 Jul 29; 346(8970):281-282.
267. Attwood JT, Yung RL, Richardson BC. DNA methylation and the regulation of gene transcription. *Cell Mol Life Sci.* 2002 Feb; 59(2):241-257.
268. Bird A. DNA methylation patterns and epigenetic memory. *Genes Dev.* 2002 Jan 1; 16(1):6-21.
269. Petronis A. The genes for major psychosis: aberrant sequence or regulation? *Neuropsychopharmacology.* 2000; 23(1):1-12.
270. Abdolmaleky HM, Yaqubi S, Papageorgis P, Lambert AW, Ozturk S, Sivaraman V, et al. Epigenetic dysregulation of HTR2A in the brain of patients with schizophrenia and bipolar disorder. *Schizophr Res.* 2011 Jul; 129(2-3):183-190.
271. Ghadirivasfi M, Nohesara S, Ahmadkhaniha HR, Eskandari MR, Mostafavi S, Thiagalingam S, et al. Hypomethylation of the serotonin receptor type-2A Gene (HTR2A) at T102C polymorphic site in DNA

derived from the saliva of patients with schizophrenia and bipolar disorder. *Am J Med Genet B Neuropsychiatr Genet*. 2011 Jul; 156B(5):536-545.

272. Guhathakurta S, Singh AS, Sinha S, Chatterjee A, Ahmed S, Ghosh S, et al. Analysis of serotonin receptor 2A gene (HTR2A): association study with autism spectrum disorder in the Indian population and investigation of the gene expression in peripheral blood leukocytes. *Neurochem Int*. 2009; 55(8):754-759.

273. Bunzel R, Blumcke I, Cichon S, Normann S, Schramm J, Propping P, et al. Polymorphic imprinting of the serotonin-2A (5-HT2A) receptor gene in human adult brain. *Brain Res Mol Brain Res*. 1998; 59(1):90-92.

274. Hazell L, Shakir SA. Under-reporting of adverse drug reactions : a systematic review. *Drug Saf*. 2006; 29(5):385-396.

275. von Knorring L, Akerblad AC, Bengtsson F, Carlsson A, Ekselius L. Cost of depression: effect of adherence and treatment response. *Eur Psychiatry*. 2006 Sep; 21(6):349-354.

276. Lesén E, Petzold M, Andersson K, Carlsten A. To what extent does the indicator "concurrent use of three or more psychotropic drugs" capture use of potentially inappropriate psychotropics among the elderly? *Eur J Clin Pharmacol*. 2009 Jun; 65(6):635-642.

277. Merlo J, Wessling A, Melander A. Comparison of dose standard units for drug utilisation studies. *Eur J Clin Pharmacol*. 1996; 50(1-2):27-30.

278. Rico-Villademoros F, Calandre EP. Reasons for prescription of serotonin receptor 2A-blocking antidepressants may confound the association between their use and the occurrence of joint disorders: comment on the article by Kling et al. *Arthritis Care Res (Hoboken)*. 2010 May; 62(5):744; author reply 744-745.

279. Blackshear MA, Martin LL, Sanders-Bush E. Adaptive changes in the 5-HT2 binding site after chronic administration of agonists and antagonists. *Neuropharmacology*. 1986 Nov; 25(11):1267-1271.

280. Roth BL, Ciaranello RD. Chronic mianserin treatment decreases 5-HT2 receptor binding without altering. *Eur J Pharmacol*. 1991 Jun 19; 207(2):169-172.

