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Personalized medicine can pave the way for the safe use of CB_1 receptor antagonists

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Antagonists of cannabinoid type-1 (CB_1) receptors have been explored as therapeutic agents for obesity and addiction. However, use of rimonabant (the first marketed CB_1 receptor antagonist) has been suspended due to its anxiogenic and depressive side effects (including suicide risk). Recent genomic studies provide evidence that variants of the CB_1 receptor gene *(CNR1)* alone or in combination with the gene of the serotonin transporter *(SLC6A4)* contribute to the development of anxiety and/or depression, suggesting that high-risk individuals could be identified through genetic testing. In this review, we argue that identification of high-risk individuals by a combination of genomic screening, previous risk phenotype, and environmental risk factors offers a promising method for the safe use of centrally acting CB_1 receptor antagonists. We summarize endocannabinoid signaling in pathways related to anxiety and depression, identify the serotonergic system as the most likely candidate to mediate the side effects of CB_1 receptor antagonists, and propose that polymorphisms in *CNR1*, *SLC6A4* and certain CYP 450 enzymes could help to identify individuals who may benefit from treatment with CB_1 receptor antagonist without psychiatric side effects.

Introduction

Some of the most promising molecules in pharmacological research of the last decade were antagonists of the cannabinoid type-1 (CB_1) receptor. This was due to their potential therapeutic effects on obesity and addictive disorders, two major public-health problems in 'developed countries'.

The CB_1 receptor is the mediator of endocannabinoids in the central nervous system (CNS). Initial neurobiological studies demonstrated that it is expressed in the axon terminals of neurons located in the cerebral cortex, basal ganglia, and limbic structures [1–3]. Detailed analyses of its physiological function became feasible after the discovery of the first selective antagonist of the CB_1 receptor: rimonabant (Sanofi-Aventis). The crucial role of the CB_1 receptor in the regulation of emotion processing, pain perception, and motivation for food intake attracted extraordinary attention. This is because extensive research suggested that CB_1 antagonists can be effective in the treatment of obesity and metabolic dysregulation [3–5], including impaired sensitivity to insulin, diabetes mellitus [6] and dyslipidemia [5,7]. There are also promising data on addictive disorders such as alcohol consumption [8,9] and tobacco dependence [10–13]. Rimonabant seemed to be revolutionary in the treatment of nicotine dependence because of its negative effect on weight gain (which is often associated with smoking cessation). Further investigations completed the possible therapeutic indications with other types of drug dependence (cannabis, cocaine, opioids) [14]; coronary artery disease [15]; hypotension/shock [16]; liver disease [17]; gastrointestinal disease [17] and arthritis [18]. The therapeutic effect of rimonabant was confirmed by the Rimonabant in Obesity (RIO) studies (RIO-Europe, RIO-Lipids, RIO-North America, RIO-Diabetes).

Rimonabant and psychiatric adverse events

Rimonabant was introduced into practice as an antiobesity agent in several countries, including those in North America and the European Union. However, the USA Food and Drug Adminstration (FDA) asked further evidence about the safety of rimonabant before approving its marketing in the USA. This is shown in the US Food and Drug Administration Endocrinologic and Metabolic Advisory Briefing Information from 2007 (available at http://www.fda.gov/ohrms/dockets/ac/07/briefing/2007-4306b1-fda-backgrounder.pdf). Even if psychiatric disorders were used as exclusion criteria, rimonabant-treated patients experienced psychiatric problems such as mood symptoms, anxiety and suicidal tendencies more frequently than placebo controls [7,19–22]. In an analysis of the data of RIO studies by the FDA, 26% of participants taking 20 mg rimonabant had some psychiatric side effects compared with 14% taking placebo. A meta-analysis of these four RIO studies suggested that patients treated with rimonabant (20 mg daily) were 2.5- and 3.0-times more

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likely to discontinue treatment because of anxiety and depression or depressive symptoms than patients receiving placebo [4].

Further investigations in which psychiatric disorders were not regarded as exclusion criteria (Strategy to Reduce Atherosclerosis Development Involving Administration of Rimonabant – The Intravascular Ultrasound Study (STRADIVARIUS); Study Evaluating Rimonabant Efficacy in Drug-Naive Diabetic Patients (SERENADE); Efficacy and Safety of Rimonabant as an Aid to Smoking Cessation With or Without Nicotine Patch (CIRRUS); An International Study of Rimonabant in Dyslipidemia With Atherogenic Risk In Abdominally Obese Patients (ADAGIO-Lipids)) showed that adverse psychiatric effects were more pronounced in the treated population versus placebo controls [6,10,15,23]. The European Union (EU) Committee for Medicinal Products for Human Use found that rimonabant doubled the risk of psychiatric disorders, and the European Medicines Agency (EMA) suspended the license of rimonabant. Sanofi-Aventis has withdrawn the product from sale worldwide, and also stopped clinical development. These events influenced the entire industrial market. The development of several other CB_1 receptor antagonists not yet approved for marketing, such as otenabant (Pfizer), which was in Phase III trials, and ibipinabant (Solvay/Bristol-Myers Squibb) and surinabant (Sanofi-Aventis), which were in Phase II trials, were interrupted because of similar preliminary clinical trial results [24,25]. In addition, a recently published report about the clinical trial of taranabant (Merck) also concluded that further development is not supported because of psychiatric side effects [26]. However, recent evidence suggests that

Table 1. Currently available pharmacogenomic tests for drugs. (see http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm and http://www.pharmgkb.org/resources/forScientificUsers/pharmacogenomic_tests.jsp)

Label	Medication	Indication	Genetic biomarker	Adversity/therapeutic effect
PGt test Required (precondition to prescription)	cetuximab	Colorectal cancer	KRAS mutation	Prediction of therapeutic effect
	Erlotinib, gefitinib	Lung cancer	EGFR deletions in exon 19 and insertions in exon 20	
	trastuzumab	Breast cancer	ERBB2 gene amplification	
	imatinib	Chronic Myelogenous Leukemia (CML)	ABL1; BCR Reciprocal translocation (9;22)(q34;q11)	
	panitumumab	Colorectal cancer	KRAS mutation	
	dasatinib	CML	ABL1; BCR Reciprocal translocation (9;22)(q34;q11)	
PGt test required in at-risk population*	carbamazepine	Epilepsy, bipolar affective disorder	HLA-B*1502	Toxic epidermal necrolysis, Steven-Johnson Syndrome, fatal
PGt test recommended	warfarin	thrombosis	CYP2C9 (CYP2C9*2, CYP2C9*3) VKOC1 (VKORC1:G-1639A)	Bleeding
	azathioprine	Renal transplanatation	TPMT	Myelotoxicity, fatal
	nilotinib	CML	UGT1A1	hyperbilirubinaemia
	abacavir	HIV-1 infection	HLA-B*5701 allele	Hypersensitivity reaction
	irinotecan	Colorectal cancer	UGTA1 polymorphisms	Neutropenia, severe diarrhea
Information-only PGt test**	metoprolol	hypertension	CYP2D6	Higher plasma level in PM
	celecoxib	arthritis	CYP2C9 variants	Higher plasma level in PMs
	thioridazine	schizophrenia	CYP2D6 variants	Toxic level of drug in PM
	clopidogrel	thrombosis	CYP2C19 variants	Cardiovascular events in PMs
	fluoxetine	depression	CYP2D6 variants	Toxic high dose in PMs
	voriconazol	mycosis	CYP2C19 variants	Toxic level in PMs
	prasugrel	thrombosis	CYP2C19 variants	UM phenotype is associated with CV events
	atomoxetine	ADHD-sy	CYP2D6 variants	PM=High plasma level
	codein sulfate	pain	CYP2D6 variants	Toxic plasma morphine (metabolite of codein) level
	capecitabine	Colorectal cancer	DHD deficiancy	stomatitis, diarrhea, neutropenia and neurotoxicity

PGt test, pharmacogenomic test; PM, poor metabolizer; UM, ultra-rapid metabolizer; DPD, dihydropyrimidine; TPMT, thiopurine S-methyltransferase; UGT1A1, uridine glucuronosyltransferase 1A1; VKOC1, vitamin K epoxide reductase; ADHD-sy, attention-deficit/hyperactivity disorder syndrome.
In the case of CYP2C19, 15–20% of Asians, 3–5% of Caucasians and Blacks are PMs whereas 30% of the population is UMs. CYP2D6-related PMs occur in 7% of the population. UMs are distributed as: 0.5–1% in Asians; 1% in Hispanics; 1–10% in Caucasians; 3% of African–Americans; Ethiopians and Arabs; and 16–28% in North Americans. Decreased activity of TPMT is present in 10% of the general population, and 0.3% have inactive TPMT. Ten percent of the North American population show decreased activity of UGT1A1 [107].

*Carrying the HLAB*1502 allele has a higher risk in the Chinese population.

**The PGt test is available on the market. Hence, it can be purchased by individuals but it is not recommended for all patients in the basic health care system.

it might be possible to determine which patients are at high risk of psychiatric side effects through detailed phenotypic assesments and genetic testing. This could herald 'personalized medicine'. It could also provide an opportunity to treat patients at a low risk of psychiatric side effects from CB_1 antagonists (~70% of the studied patient populations) for conditions such as obesity and metabolic dysregulation that have, as yet, no effective treatment.

Here we review the current possibilities for pharmacogenomic tests (PGts). That is: single nucleotide (SNP) or other polymorphisms in candidate genes (including those of the CB_1 receptor), the serotonin transporter (SERT) and key CYP 450 enzymes. Furthermore, the molecular and genetic aspects of CB_1 receptor antagonist-associated anxiety and depression are also discussed.

Personalized medicine based on genotypes

Management of the adverse effects of a drug is complex and often difficult challenge. Personalized medicine includes prediction of therapeutic effects or side effects before introduction of a drug by using biomarker tests. An increasing amount of data is available about genetically determined adverse events of certain (sometimes frequently used) drugs. Some of these side effects can be predicted by PGts, thereby saving the drug from withdrawal from the market. Currently, side effects can be identified mainly by those PGts which are associated with an increased or decreased level of the drug in plasma due to a genetically altered function of metabolizing enzymes. For several drugs, the use of PGts before medical treatment is required or recommended by the official label of medicinal products (Table 1). Variations in pharmacodynamic effects are more numerous, and these types of PGts are under development due to new biotechnological possibilities. For example, two promising PGts are under development for CNS disorders. One test is the genotyping of two SNPs in the gene HLADQB1 in patients who are candidates for the antipsychotic drug clozapine. Patients with the high-risk alleles are more likely to develop agranulocytosis (a life-threatening depletion of white blood cells). The second test is the PhyzioType™ System, which detects genetic markers associated with an increased risk for the metabolic syndrome in patients taking antipsychotic medications [27].

Genetic association of the CB_1 receptor gene (*CNR1*) with anxiety and depression

Depression and anxiety are complex neuropsychiatric disorders. Several genes interact with each other and with environmental factors, and are involved in the pathophysiology of these disorders. This also means that diagnostic categories are arbitrary; depressive and anxiety traits show Gaussian distribution in the population. Thus, depression and anxiety are present in the population as quantitative traits. If the symptoms exceed a pre-defined threshold because of additional stressors such as negative life events or drugs, these can be diagnosed as disorders [28]. It is unsurprising that patients treated with rimonabant who have a depressive episode in their history are more likely to report anxiety or depression as a side effect than those with a negative history [29,30].

In line with the quantitative trait hypothesis mentioned above, psychiatric side effects have also been reported (albeit with less frequency) if psychiatric disorders are used as exclusion criteria [29]. This suggests that the side effects of the CB_1 receptor antagonist are based on pre-existing traits rather than producing disorders in low-risk individuals [29]. In fact, multiple phenotypes are present in the general population that carry similar genetic predisposition to depression or anxiety disorders. This comprises three groups. First are individuals with a definitive diagnosis of a psychiatric disorder (this group was excluded from treatment with a CB_1 antagonist in the RIO studies). Second are individuals with no current psychiatric symptoms but who have a history of depression or anxiety that never received a diagnosis (even though it could be explored with a relevant test or interview). Third are individuals who do not have a current episode or history of major psychiatric episodes but in whom it will be manifested by a provocation factor such as the CB_1 antagonist. These phenotypes together create the 'at-risk' population in the use of CB_1 antagonists.

Thus, it would be highly important to identify those patients predisposed to the anxiety- and mood-related side effects of CB_1 receptor antagonists by simultaneously screening for depression-related traits and environmental and genetic risk factors. Indeed, predisposition to anxiety or depression is genetically determined; their heritability is 32–37% [31]. Thus, a biologically relevant screening test for treatment with rimonabant could be a PGt containing candidate polymorphisms. CB_1 receptor antagonists act through a well-characterized biological pathway (Figure 2), so genetic variants involved in this pathway are plausible candidates. Here, we discuss the relevance of the strongest candidate: *CNR1*.

Recent research has identified a significant genetic SNP x SNP interaction (epistasis) of *CNR1* on high neuroticism and low agreeableness. These are personality traits that predispose individuals for several psychiatric disorders (e.g. depression, anxiety). The effects of *CNR1* haplotypes were also shown to be significantly associated with current depression score, and this association was dependent upon negative life events, suggesting an interaction between the gene and the environment [32]. Our research team investigated the interaction between *CNR1* promoter variants and the functional polymorphism of the serotonin transporter gene *5-HTTLPR* on trait anxiety and temperament measures in a sample of the general population [33]. The *5-HTTLPR* x *CNR1* promoter interactions on trait and temperament measures of anxiety were highly significant. The risk to score higher on the anxiety scale of the Brief Symptoms Inventory was 4.6-fold greater in persons who were homozygous 'GG' of the rs2180619 promoter polymorphism in *CNR1* and also homozygous 'SS' of *5-HTTLPR*. The 'G' allele of rs2180619 (together with the previously described "TGC" alternative promoter) is associated with low expression of the CB_1 receptor that produces an attenuated suppression and thus an elevation of 5-hydroxytryptomine (5-HT; serotonin) release from the terminal. The 'SS' genotype is associated with low expression of the 5-HT transporter, and thus less efficient 5-HT reuptake and termination of the 5-HT effect. The combination of these

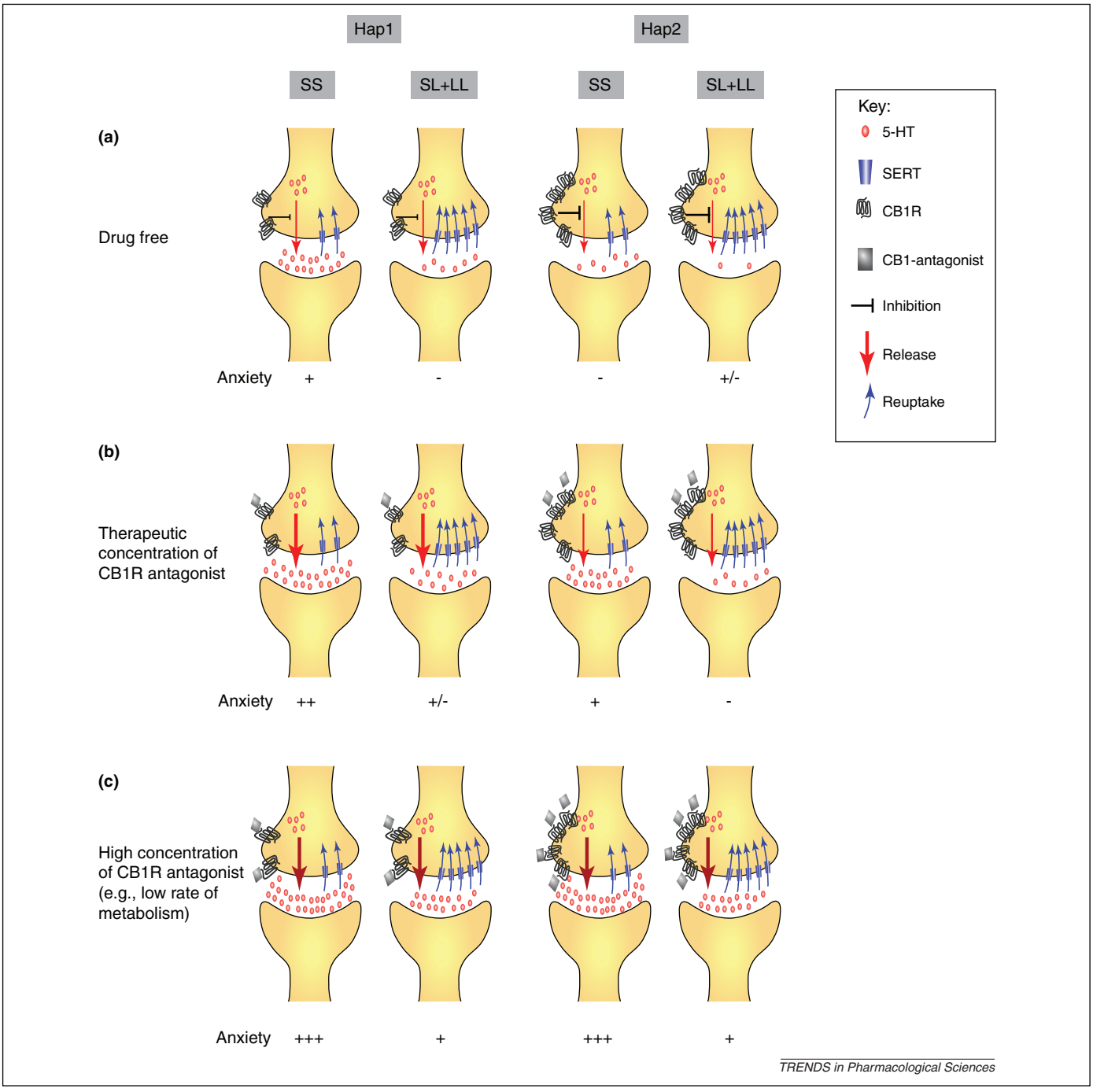

Figure 1. Genetic model on the interaction between CNR1 and 5-HTTLPR in association with anxiety.
Synaptic 5-HT concentration and having an anxious phenotype can be influenced by genetic variants of the CB_1 receptor and SERT, as demonstrated by our previous study [33]. In general, anxiety is dependent upon extracellular 5-HT concentration. In drug-free conditions, higher- and even lower-than-normal extracellular 5-HT levels might lead to increased anxiety. Haplotype 1 (Hap1) of *CNR1* is associated with lower expression of CB_1 receptors (which results in weaker inhibition of 5-HT release) whereas Haplotype 2 (Hap2) is associated with higher CB_1 receptor expression (and stronger inhibition of 5-HT release). In L allele carriers of *5-HTTLPR*, 5-HT reuptake is more pronounced than in the SS genotype because of elevated SERT expression (**a**). Anxiety-related side effects of treatment with CB_1 receptor antagonists are dependent upon different genotypes and drug dose. Therapeutic drug concentrations of CB_1 receptor antagonists might cause anxiety in SS carriers of haplotype 1 and, to a lesser extent, SS carriers of haplotype 2, because of elevated extracellular 5-HT concentrations. In the case of L allele carriers, a slight increase in anxiety might occur with haplotype 1 but, in haplotype 2 carriers, CB_1 receptor antagonists might decrease trait anxiety symptoms and over-reaction of stressful life events (**b**). In the case of very high doses of CB_1 receptor antagonists (e.g. in the slow metabolizer phenotype; liver problems; metabolic drug interactions) anxiety might be increased in all genotype carriers because of blockade of CB_1 receptors. However, L carriers might be protected against extremely high 5-HT concentration and associated untolerable anxiety by the increased expression of SERT (**c**). Part A of the figure is modified from Lazary *et al.* (2009) [33].
''–'' means normal level of anxiety; ''+'', ''++'', and ''+++'' mean higher than normal anxiety in increasing order.

genetic variants results in an extremely high synaptic 5-HT concentration and, based on our studies, an increase in anxiety (Figure 1) [33]. The association of the 'SS' genotype with high anxiety and elevated extracellular 5-HT concentration is further supported by the data that agitation as an adverse reaction to SSRI treatment has a 9.5-fold greater incidence in 'SS' carriers compared to SL plus LL carriers [34].

Further evidence was provided for the role of *CNR1* in depression and anxiety by the work of Domschke and colleagues [35]. Their results showed that the response of patients with major depression to antidepressant treatment (particularly in women with co-morbid high anxiety) was influenced by *CNR1* variants. Furthermore, the risk allele that predicted poorer outcome to treatment was also associated with decreased activation in the brain upon viewing happy faces [35] (an important neurocognitive marker of a depressive phenotype) in a functional magnetic resonance imaging (fMRI) study. A recently published study suggested that variants in *CNR1* (and also in fatty acid amide hydrolase (FAAH)) genes confer risk for mood disorders [36].

Endocannabinoid signaling in pathways related to anxiety and depression

Anxiety, depression and suicide are highly co-morbid and share common etiologic, genetic and neurobiological risk factors. Among them, anxiety has the highest prevalence in the population (although it tends to transform into depression or mixed anxiety-depression) [31,37]. Depression is the most common psychiatric disorder that is associated with completed suicides; nevertheless, additional factors (e.g. impulsivity, aggression, seasons, sex, cultural norms) have significant contributions [38]. Meta-analyses of RIO studies show that the most consistent side effect of rimonabant is anxiety. Thus, we propose to use anxiety (the most investigated behavioral model for the effect of CB_1 receptor antagonists) as a primary endpoint.

Endocannabinoids are synthesized in postsynaptic neurons constitutively or after neurotransmitter stimulation by cleavage of membrane phospholipids, and are released into the synaptic cleft, where they function as retrograde messengers [1–3]. Leaving the postsynaptic neurons, they travel through the synaptic cleft and activate presynaptic CB_1 receptors and inhibit the release of excitatory and inhibitory neurotransmitters (see below). Inhibition of the endocannabinoid system with CB_1 receptor antagonists could then increase neurotransmitter release and cause anxiety and. as a consequence, depression and possibly suicide.

CB_1 receptors are expressed at a very high level in gamma-aminobutyric acid (GABA)ergic neurons, whereas they are expressed at a low-to-moderate level in glutamatergic neurons [1–3]. Thus, an increase in GABA release by CB_1 receptor antagonists could occur, but this, like the GABA modulator benzodiazepine anxiolytics, would cause a decrease in fear and anxiety. By contrast, CB_1 receptor antagonists such as rimonabant tend to increase anxiety. This suggests that CB_1 receptors located at GABAergic terminals are not responsible for the anxiogenic effects of CB_1 receptor antagonists.

The increased release of glutamate might contribute to the anxiogenic effect of CB_1 receptor antagonists because, for example, ketamine (a non-competitive *N*-Methyl-D-aspartic acid (NMDA) receptor antagonist) infused intravenously causes a rapid antidepressant effect in individuals with treatment-resistant depression and also in animal tests [39]. However, several contradictory studies have been observed, and clinical data to support the role of glutamate release in the action of CB_1 receptor antagonists in anxiety and depression are scarce [40–42].

By contrast, several lines of evidence suggest that monoamines such as 5-HT are relevant candidates for the mediation of the anxiogenic effect of CB_1 receptors (Figure 2). First, 5-HT transporter knockout (KO) mice and CB_1 receptor KO mice demonstrate increased anxiety and fear [40,43]. CB_1 receptors are present and function on 5-HT terminals [44] and, as described above, human genetic studies provided evidence for the interaction of the CB_1 receptor gene, the SERT gene, and anxiety [33]. In addition, increase in the synaptic/extracellular concentration of 5-HT (e.g. after acute selective serotonin reuptake inhibitor (SSRI) challenge) causes anxiety-like effects in rodents and mainly in 'SS' carrier humans [45–47,34]. This effect is blocked by selective 5-HT2C receptor antagonists in rats [45,46] and is absent after chronic treatment with SSRIs [47], suggesting the role of this receptor subtype in the process..

The 5-HT2C receptor agonist *meta*-chlorophenypiperazine (*m*-CPP) causes anxiety in humans and rodents [46,48–51]. This effect is blocked by selective 5-HT2C receptor antagonists [46]. 5-HT2C receptors within the basolateral amygdala induce acute fear-like responses in an open-field arena [52] and activation of the 5-HT2C receptor is involved in the enhanced anxiety in rats after single prolonged stress [53]. In addition, anxiety-like behavior observed after uncontrollable traumatic stress in rodents is mediated by exaggerated 5-HT acting at 5-HT2C receptors in the basolateral amygdala [54]. Human fMRI studies showed that the effect of *m*-CPP is associated with activation of the amygdala [55]. Rat fMRI experiments provided evidence that the anxiogenic doses of *m*-CPP are mediated by the activation of limbic brain areas [56]. Agonists of central 5-HT2C receptors (including *m*-CPP) cause activation of the sympathoadrenal system and the hypothalamic–pituitary axis (HPA) in humans and rats, and involvement of the hypothalamic paraventricular nucleus and corticotropin releasing hormone (CRH) in the latter have also been proven [50,57–59]. Furthermore, 5-HT2C receptor KO mice display blunting of amygdala CRH neuronal activation in response to anxiety stimuli [60], and overexpression of 5-HT2C receptors in the forebrain leads to elevated anxiety in transgenic mice [61]. Taken together, these studies strongly suggest that the 5-HT receptor that mediates anxiety and fear after elevated 5-HT concentration is probably the 5-HT2C receptor subtype.

Further evidence for the involvement of 5-HT reuptake and synaptic/extracellular 5-HT concentration in fear processing is suggested by the fact that amygdala activation to fearful faces is associated with the 5-HTTLPR S allele [65] (see also Table 2). The role of endocannabinoids and CB_1 receptors in this process is further suggested by data showing that activation of receptors with Gq/11-mediated signal transduction causes the *in-situ* synthesis and release of 2-AG through activation of phospholipaseCβ and diacylglycerol lipase [63,64]. In addition, antidepressant treatments (including inhibition of 5-HT reuptake and thus 5-HT2C receptor activation) alter CB_1 receptor expression in limbic brain regions involved in depression and anxiety (e.g. hippocampus, amygdale) [65]. Under normal,

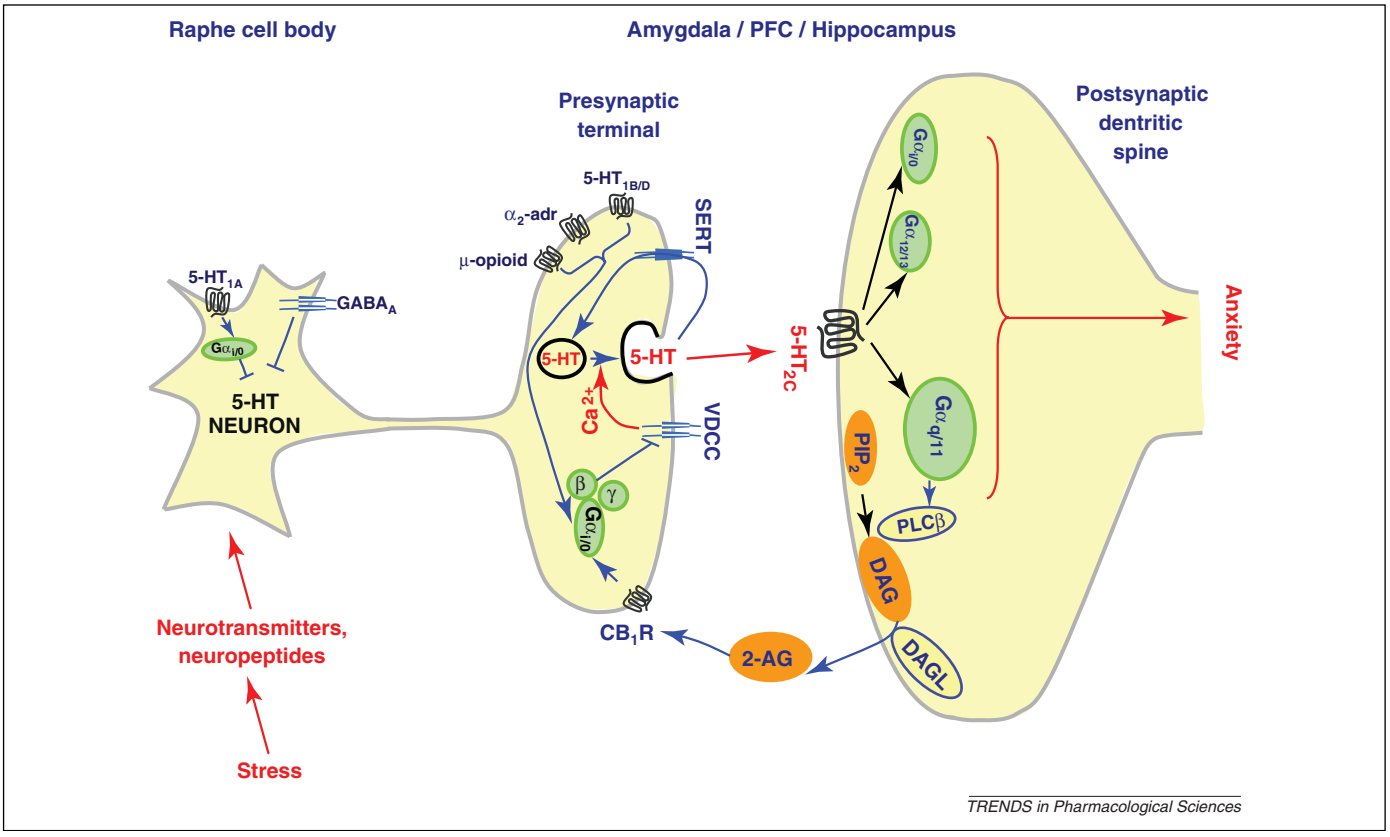

Figure 2. Mechanism of the anxiogenic effect of CB_1 receptor antagonists.
Stress stimulates serotonergic neurons of raphe nuclei projecting to the amygdala, prefrontal cortex (PFC) or hippocampus, thereby causing anxiety by stimulation of 5-HT2C receptors. Acute 5-HT stimulation causes anxiety by this mechanism. Paradoxically, chronic elevation of extracellular 5HT concentration in the brain has an antidepressant effect because it downregulates 5-HT2C and other 5-HT receptors, and desensitizes their signaling. This explains the antidepressant and anxiolytic effects of chronic (≥3 weeks) treatment with SSRIs, as well as the acute, transient anxiogenic-like (e.g. in anxiety tests in rodents, and agitation or jitteriness in patients) side effects of SSRIs that may cause a recution in treatment compliance [46] and which is also associated with the genetic polymorphism of the SERT gene, i.e. the SS allele of *5-HTTLPR* [34]. Presynaptic CB_1 receptors have tonic inhibitory activity on 5-HT release due to constitutive receptor activity and/or endocannabinoid (including 2-arachydonoyl-glycerol (2-AG)) release. 5-HT activates postsynaptic 5-HT2C receptors, which stimulate $G_{q/11}$ proteins and cause phospholipase Cβ-mediated diacylglycerol (DAG) production [63]. DAG lipase can convert DAG to 2-AG, which can cause retrograde stimulation of CB_1 receptors, leading to inhibition of Ca^{2+} influx and 5-HT release. Therefore, CB_1 receptor antagonists stimulate 5-HT release, and cause anxiety by stimulating 5-HT release and activation of 5-HT2C receptors. Anxiogenic and anxiolytic mechanisms are shown in red and blue, respectively.

drug-free conditions, the *in situ* synthesis and release of 2-AG could counteract the decrease in SERT function. However, CB_1 receptor antagonists (particularly at high concentrations) enhance the release of 5-HT and thus extracellular 5-HT concentration in the forebrain and block the enhanced inhibitory actions of 2-AG as described above [66,67]. Furthermore, the persistent lack of CB_1 receptor activity impairs serotonergic negative feedback and functionality of 5-HT1 and 5-HT2A/C receptors in CB_1 receptor KO [68,69], suggesting developmental and/or adaptive changes in the serotonergic system due to the lack of functional CB_1 receptors. A model including the role of the 5-HT transporter, extracellular 5-HT concentration, 5-HT2C receptor, endocannabinoids, CB_1 receptor and the process that explains the role of CB_1 receptor antagonists on anxiety is shown in Figure 2.

The contributing role of other 5-HT receptors and other neurotransmitters and receptors cannot be excluded [70]. Some of them are shown also in Figure 2. Central noradrenalin might be another 'suspect' because there is evidence that this neurotransmitter also plays a significant part in reactions to stress reaction. Furthermore, an interaction between the CB_1 receptor and α1 adrenergic receptor might be possible because the latter increases the concentration of 2-AG, and thus also induces autoinhibition through CB_1 receptors.

Other candidate genes associated with anxiety, depression or suicide

SERT gene (SLC6A4)

A depression-related phenotype (depression, anxiety, suicidal behavior) is a multifactorial, polygenic condition, so several genes are candidates for a PGt (Tables 2 and 3). One of the most studied genes in anxiety- and depression-related phenotypes is the serotonin transporter gene (*SLC6A4*). Although some well-powered meta-analyses concluded that *SLC6A4* variants are not significantly associated with anxiety and depression disorders in a direct way, especially in case–control designs [71–74] (see also Table 2), the unambiguous role of the serotonin transporter in the regulation of emotional behavior is supported by genetic studies of rhesus monkeys, behavioral investigations of *SLC6A4* KO mice, and functional neuroimaging studies in humans [62,75]. With respect to phenotypes, lessons from genome-wide association research show that psychiatric diagnostic categories overlap and do not represent biological pathways [76–78]. Furthermore, these studies emphasize that the heritability of complex traits and

Table 2. Association of key pharmacogenetic candidates with depression related phenotypes and drug responses.

	Neuroticism	Depression	Suicidal behavior	Pharmacogenetic data
CNR1	- haplotyoic association and epistatic interaction [32] - association in interaction with SLC6A4 on trait and temperament measures of anxiety [33]	-direct association [36] -association in interaction with negative life events [32]	no data	influence on antidepressant therapy response [35]
SLC6A4	-trait measures:direct association [72,79,80] -fMRI and neuroimaging studies: exaggerated threat-related amygdala reactivity in healthy volunteers and also psychiatric patients; altered functional coupling between amygdala and prefrontal cortex, and decreased gray matter volume in both (for review see [81])	- inconsistent data about direct association (for reviews see. [109]	-involved in suicide related traits [92,93]	influence on SSRI antidepressant treatment response and side-effect [34,86]
		-association in interaction with negative life events (for reviews and metaanalyses see [81] and [[83])		
		-epistatic association in interaction with negative life events [84]		
CYP2D6 and CYP2C19	no data	no data	no data	influence toxic effect of antidepressant s [85]

Neuroticism or negative affectivity is a latent personality trait including overreaction of stressful or even neutral events. Haplotyptic association and epistatic interaction mean different allelic effect on phenotype. In these studies several polymorphisms of one or more genes are investigated in association with phenotype. Further details and references could be found in the text.

common disorders such as depression and anxiety is due to multiple genes of relatively small effect size [28]. Consequently, the trait of neuroticism (including over-reaction of stressful or even neutral events) was accepted as a more appropriate phenotype in genetic studies, and its association with the presence of the S allele was demonstrated by several consequent reports [73,79,80]. In human fMRI studies, an association of exaggerated threat-related amygdala reactivity in healthy volunteers and psychiatric patients, and altered functional coupling between the amygdala and prefrontal cortex with SLC6A4 variants, has been described (for review, see [81]) (Table 2).

Table 3. Association of other candidate genes with anxiety, depression and suicide.

Genes	Anxiety	Depression	Suicide
ADORA2A	Positive	No data	No data
BDNF	Positive	Mixed	Positive
COMT	Positive	Mixed	Positive
CREB1/CREM	Positive	Positive	No data
CRH/CRHR	Positive	Positive	Partly (in alcoholics)
DRD2	Positive	Mixed	Mixed
DRD4	Positive	Positive	No association
HTR1A	Positive	Positive	Mixed
HTR1B	Positive	Mixed	Partly (in alcoholics)
HTR2A	Positive	No association	No association
MAOA	Positive	Mixed	Mixed
MTHFR	No association	Positive	No data
P2RX7	No association	Positive	No data
SLC1A1	Positive	No data	No data
SLC6A2	Positive	Positive	No data
TPH1	No association	Mixed	Mixed
TPH2	Positive	Mixed	Mixed

Associations between candidate genes and phenotypes are evaluated by meta-analyses (if available). A 'positive' association between gene and phenotype was reported; 'mixed' conflicting results about the association have been published. References are stated in the text.

In studies on possible interaction of the *SLC6A4* gene and *CNR1* on trait anxiety and temperament, no significant individual effect of these genes was observed [33]. These conflicting results could be explained by the continuous counteracting effects mediated by CB_1 receptors, as described above (see also Figure 2), difficulties in phenotype measurement, and by the modifying impact of environmental factors on the appearance of genetic effects ('gene–environment interactions' (G × E)). The effects of *SLC6A4* and stressful life events in association with depression were first demonstrated by Caspi *et al.* [82]. In that study, the number of negative life events was associated with the number of major depressive episodes and depressive symptoms, and the effect was much stronger in *5HTTLPR* short allele carriers than in long allele carriers. Attempts to replicate this finding have shown conflicting results depending on the method used for evaluation of phenotypes and environmental factors. However, a more extensive recent meta-analysis of 54 studies confirmed the significant effect of interaction on depression [83]. Haplotype analyses of the SLC6A4 in interaction with life-threatening events suggests that a significant gene–gene–environment interaction influences depressive phenotype [84]. In addition, the significant *CNR1* haplotype association with depressive symptoms described above became non-significant after controling for recent negative life events scores. These data suggest that the *CNR1* gene

(similarly to *SLC6A4*) modifies the depressogenic effect of life events [32] (Table 2).

In contrast to the genotype–phenotype association investigations, evidence from pharmacogenomic studies suggest that drug effects (even those related to anxiety and depression) are more closely associated with already identified genes (including *SLC6A4*) [34,85]. Conclusions from pharmacogenetic reviews on antidepressants suggest that S allele carriers show a significantly worse response and remission rate during SSRI treatment compared with LL genotype carriers [86,87]. Less effective treatment can be explained by increased side effects and therefore worse compliance, which was also associated with the carrying of S alleles [34,86]. Indeed, anxiety-related adverse reactions observed during SSRI treatment are much greater and more frequent in the 'SS' 5HTTLPR carriers [34], suggesting that increased synaptic serotonergic level can cause this side effects. Similarly to the SERT, the CB1 receptor has also important role in the regulation of synaptic and extracellular serotonin concentration, thus disinhibition of 5-HT release by this receptor subtype can also cause anxiety (Figures 1, 2 and Table 2).

CRH

Genes related to CRH might also be involved in depression or anxiety. Transgenic mice that overproduce CRH are an important model of anxiety and depression. Consequently, corticotrophin-releasing hormone receptor 1 gene (*CRHR1*) KO mice show diminished anxiety [88]. Investigations of human genetic variants confirmed the role of *CRH* and *CRHR1* genes on anxiety and depression [75,88], although genome-wide association studies (GWAS) failed to show an effect for *CRHR1* (Table 3).

Additional candidate genes

Replicated positive results are available on catechol-*O*-methyltransferase (*COMT*) and dopamine receptor D4 (*DRD4*) receptor genes in association with anxiety and depression [89,90]. Currently promising new candidates are voltage-gated calcium channel alpha 1 subunit (*CACNA1*) and kainate receptor 7 (*GLUR7*) genes in association with depression [76,91].

Genes associated with suicidal behavior

Genetic results related to suicidal behaviour are less consistent due to a much more complex and heterogeneous phenotype. Genetic studies in animals use the paradigms of anxiety or depression, but suicide itself can be investigated only in human studies. It is plausible that certain genes contributing to depression might be involved in suicidal behavior because most cases of suicide can be interpreted as a consequence of depression [38]. However, a minority of patients with depression commit suicide, suggesting that suicidal behavior is determined by several additional factors beyond depression. It is noteworthy that replicated results reported an association between suicide and *SLC6A4*, *COMT* and *tryptophan hydroxylase* (*TPH*) *genes* [92,93]. In the case of *SLC6A4,* detailed analyses suggested that it shows an association not only with suicidal behavior, but also with suicide-related endophentoypes and traits (e.g. impulsive and aggressive traits; anger-related traits; neuroticism and anxiety-related traits) [93]. Interestingly, some genes investigated in depression have not yet been studied in suicide (Table 3).

P450 enzyme polymorphisms

Rimonabant is metabolized by cytochrome P450 in the liver, and it might interact with other drugs metabolized by CYP2A6, 2C9, 2C19 and 3A4 [94,95]. Psychiatric side effects were shown to be dose-dependent in rimonabant-treated patients, so increased adversity may be caused by a markedly elevated level of the drug in plasma related to a previously described enzyme defect ('slow metabolizer'). Therefore, genetic screening of P450 enzyme polymorphisms could be applicable to predict psychiatric side effects (Table 2). In addition, genetic variants underlying the pharmacodynamic effects of rimonabant based on recently published data on CB_1 and serotonin transporter genes [32,33] (as described above and shown in Figure 1) are also promising.

Summary

Based on the literature, the role of CB_1 gene (*CNR1)* variants is evident in mediating the effect of CB_1 receptor antagonists and also in anxiety-related phenotypes. The relevancy of the SERT gene (*SLC6A4)* in depression, anxiety and suicide-related traits as well as in drug-induced side effects is based upon this premise. The latter is related to the increase in extracellular concentration of 5-HT, which has been shown to be regulated by SERT and the CB_1 receptor. Thus, the most important candidates for a screening test of CB_1 receptor antagonist-related psychiatric side effects are the *CNR1* and *SLC6A4* genes [32,33]. Further investigations are required for a complex pharmacogenomic model. Importantly, the risk population could be characterized more precisely by including the previous risk phenotype (e.g. family and individual history of depression or anxiety disorder or suicide; neurotocism, rumination) and risk environmental factors (e.g. childhood abuse, recent life stress) in addition to genetic tests.

Concluding remarks

CB_1 receptor antagonists have been approved as effective agents for treating obesity and other metabolic disorders, but their clinical introduction was unsuccessful due to their psychiatric side effects. According to a recently published meta-analysis, obesity increased the risk of the onset of depression (odds ratio (OR) = 1.55) and depression increased the chances for developing obesity (OR = 1.58) [96]. Other data suggest that 50% of patients who are looking for antiobesity treatment report depression symptoms in general clinical practice [97,98]. These findings suggest that persistent successful weight loss not only prevents the somatic effects associated with being overweight, but could also lead to improvement in mood symptoms.

It is important to consider that a risk for psychiatric side effects does not preclude successful use of a drug. Several pharmacological agents are frequently used despite having psychiatric side effects. Increased depression, anxiety or suicidal risk are associated with the use of corticosteroids, interferon-alpha, interleukin-2, gonadotropin-releasing

hormone agonists, mefloquine, progestin-releasing implanted contraceptives, propranolol, isotretinoin, montelukast, varenicline and oseltamivir phosphate [99–104]. All of these medications are labeled by a warning box detailing psychiatric side effects. Among those interferon-alpha-induced depression has been tested and shown to be associated with the S allele of the 5-HTTLPR [107,108]. These data suggest that introduction of personalized medicine is possible and could be beneficial also for these drugs.

Genetic and clinical studies in populations suggest that neuroticism is associated with CB_1 receptor gene (*CNR1)* variants [32]. An even stronger association appeared between the promoter regions of *CNR1* in interaction with the SERT gene (*SLC6A4*) in the explanation of anxiety [33]. Low expression of the *SERT* and CB_1 receptor was associated with high trait and temperament anxiety. This suggested that 'SS' carriers of *5-HTTLPR*, the SLC6A4 promoter polymorphism, are especially vulnerable to CB_1 receptor antagonist-induced psychiatric symptoms (including anxiety and depression). Furthermore, the side effects of rimonabant were clearly dose-dependent, so the genetic polymorphisms of metabolizing CYP genes could also help in the identification of vulnerable populations.

Although the promising cardiometabolic effects of peripherial CB_1 receptor antagonists recently appeared in the literature in rodent studies, it seems that significant reductions in body weight can be achieved only by central antagonists of the CB_1 receptors [105,106]. These facts suggest that further investigations of central CB_1 receptor antagonists with circumspect designs and analyses of genomic factors could lead to safe and efficient treatment of obesity as well as related high blood pressure and metabolic problems.

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