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The Role of Muscarinic Receptors in the Pathophysiology of Mood Disorders: A Potential Novel Treatment?

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Abstract: The central cholinergic system has been implicated in the pathophysiology of mood disorders. An imbalance in central cholinergic neurotransmitter activity has been proposed to contribute to the manic and depressive episodes typical of these disorders. Neuropharmacological studies into the effects of cholinergic agonists and antagonists on mood state have provided considerable support for this hypothesis. Furthermore, recent clinical studies have shown that the pan-CHRM antagonist, scopolamine, produces rapid-acting antidepressant effects in individuals with either major depressive disorder (MDD) or bipolar disorder (BPD), such as bipolar depression, contrasting the delayed therapeutic response of conventional mood stabilisers and antidepressants. This review presents recent data from neuroimaging, post-mortem and genetic studies supporting the involvement of muscarinic cholinergic receptors (CHRMs), particularly CHRM2, in the pathophysiology of MDD and BPD. Thus, novel drugs that selectively target CHRMs with negligible effects in the peripheral nervous system might produce more rapid and robust clinical improvement in patients with BPD and MDD.

Keywords: Bipolar disorder, cholinergic system, CHRM2, major depressive disorder, mood disorders, muscarinic receptors.

INTRODUCTION

Major depressive disorders (MDD) and bipolar disorders (BPD) are psychiatric illnesses characterized by inappropriate control of mood or emotions. The causes of MDD and BPD are not known, but it is now thought a combination of genetic, environmental and biochemical factors may contribute to the onset of MDD [1, 2] and BPD [3, 4]. MDD, also called unipolar depression or clinical depression, has a 12 month prevalence rate of over 6.5% and a lifetime prevalence of approximately 16% [5]. The disorder has wide reaching effects on the sufferers' lives, affecting personal and social relationships at work or school. BPD, formerly referred to as manic depression, is the second major mood disorder and is characterised by cyclic episodes of depression and mania. According to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) [6], there are four subtypes; BPD-I, BPD-II, cyclothymia and bipolar disorder not otherwise specified (BPD-NOS). The lifetime prevalence rates amongst the disorder subtypes are 0.6% for BPD-I, 0.4% for BPD-II, and 1.4% for sub-threshold BPD, which is characterized by symptoms of hypomania without a depressive episode, with a 2.4% prevalence rate for bipolar spectrum disorder (BPS) including four subtypes of BPD [7].

Current antidepressant drugs, mood stabilisers and antipsychotics can alleviate the symptoms of mood disorders in some people but in many cases treatment is only partially effective with many sufferers being refractory to treatment with current drugs [8, 9]. Therefore, there is an urgent need to discover mechanisms that may be involved in the etiology of mood disorders which are suitable for targeting by therapeutic agents. Recent clinical studies, which show that the pan-muscarinic receptor antagonist, scopolamine [10], elicits a rapid anti-depressant response in patients with MDD and BPD [11, 12], highlight a growing body of evidence suggesting the cholinergic system plays an important role in the pathophysiology of mood disorders [13-16] and may present a novel target for drug development. The question arises why the muscarinic receptor antagonist appears to have some antidepressant effects in clinical studies. Thus, in this review, we highlight the potential involvement of muscarinic cholinergic receptors in the pathophysiology of MDD and BPD and review data that suggests they may be a viable therapeutic target.

MUSCARINIC CHOLINERGIC SYSTEM IN THE CENTRAL NERVOUS SYSTEM

Acetylcholine (ACh) plays an important role as a neurotransmitter in the central and peripheral nervous systems. ACh binds to two classes of receptors [17], the muscarinic cholinergic receptors (CHRMs), which are metabotropic receptors, and the nicotinic cholinergic receptors (CHRNs), which are ligand gated ion channels. Both CHRMs and CHRNs have been acknowledged to play crucial roles in mood disorders. The possible role of CHRNs in mood disorders has been reviewed elsewhere [18, 19], hence this review will focus on CHRMs.

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CHRMs are a family of seven-transmembrane domain receptors, consisting of five receptor subtypes (CHRM1-5). As members of the G-protein coupled receptor (GPCR) superfamily, they associate with heterotrimeric G-proteins to translate the extracellular signal into an intracellular signal transduction cascade [20, 21] (Fig. **1**). In general, the canonical downstream signalling of CHRM1, 3 and 5 receptors, which are coupled to $G_{q/11}$ class of G-proteins, is initiated by activation of a Gα subunit protein [22, 23]. Phospholipase C-β (PLC-β), activated by the Gα subunit once it dissociates from the Gαβγ protein complex, hydrolyses phosphatidylinositol 4,5-bisphosphate (PIP_2) into two second messengers, inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) [22, 24]. The IP_3 binds to inositol 1,4,5-trisphosphate receptors (IP_3R) on the endoplasmic reticulum and induces their opening, causing an influx of Ca^{2+} into the cytoplasm [25]. The other second messenger, DAG, activates several isoforms (α, β, γ, δ, ε, η and θ) of protein kinase C (PKC) [26] which have different distributions or kinetics and thus different functions. Activated PKC can participate in a negative feedback of PLC-β signal transduction [27, 28] as well as activating other intracellular proteins, including the phosphorylation of protein kinases or transcription factors. By contrast, CHRM2 and CHRM4 receptors are canonically coupled to G proteins of $G_{i/o}$ class [21, 22]. The activated Gα subunit, released from Gβγ, inhibits adenylate cyclase (AC), which is responsible for catalysing the conversion of ATP to cAMP [29, 30]. Decreased intracellular cAMP levels in the cytoplasm induces the inactivation of protein kinase A (PKA), regulating ion channel activity, either opening Ca^{2+} and K^+ or closing the Na^{2+} channels in the plasma membrane. Consequently, inactivated PKA decreases the rate of internalization of Ca^{2+} into the cell and increases outward K^+ currents [31, 32].

The CHRMs have distinct functions in the central nervous system (CNS). Pharmacological research with agonists, antagonists and allosteric modulators has significantly contributed to defining the roles of specific muscarinic receptors [14, 33-35]. Gene knockout (KO) studies have also greatly advanced our understanding of the functional roles and the signalling mechanisms of CHRMs in the central cholinergic system [36]. From such studies, it has been shown that CHRM1 and CHRM3 act as postsynaptic receptors in the CNS, where they are mainly localized in the cortex and hippocampus, and play an important role in cognitive function [37]. In humans, CHRM2 and CHRM4 are reported to act as presynaptic autoreceptors in the cerebral cortex [38], caudate-putamen (CHRM4) [39] and hippocampus (CHRM4) [40], where they inhibit the release of ACh. By contrast, KO studies suggest murine CHRM2 is the cholinergic autoreceptor in the cerebral cortex and hippocampus, whilst CHRM4 acts as an autoreceptor in the striatum [41]. Finally, the distribution of CHRM5 is restricted to the hippocampus and substantia nigra [42], where they are involved in modulation of dopamine release [42, 43].

MUSCARINIC RECEPTORS IN MOOD DISORDERS

Cholinergic Hypothesis of Mood Disorders

Several lines of evidence support the involvement of the cholinergic system in mood disorders. In 1950, Rowntree showed that the organophosphorus insecticide diisopropyl-fluorophosphate (DFP), a cholinesterase inhibitor, that increases synaptic ACh levels, caused a characteristic depressive effect in healthy people and decreased manic symptoms in patients with mania [44]. In 1961, Gershon and

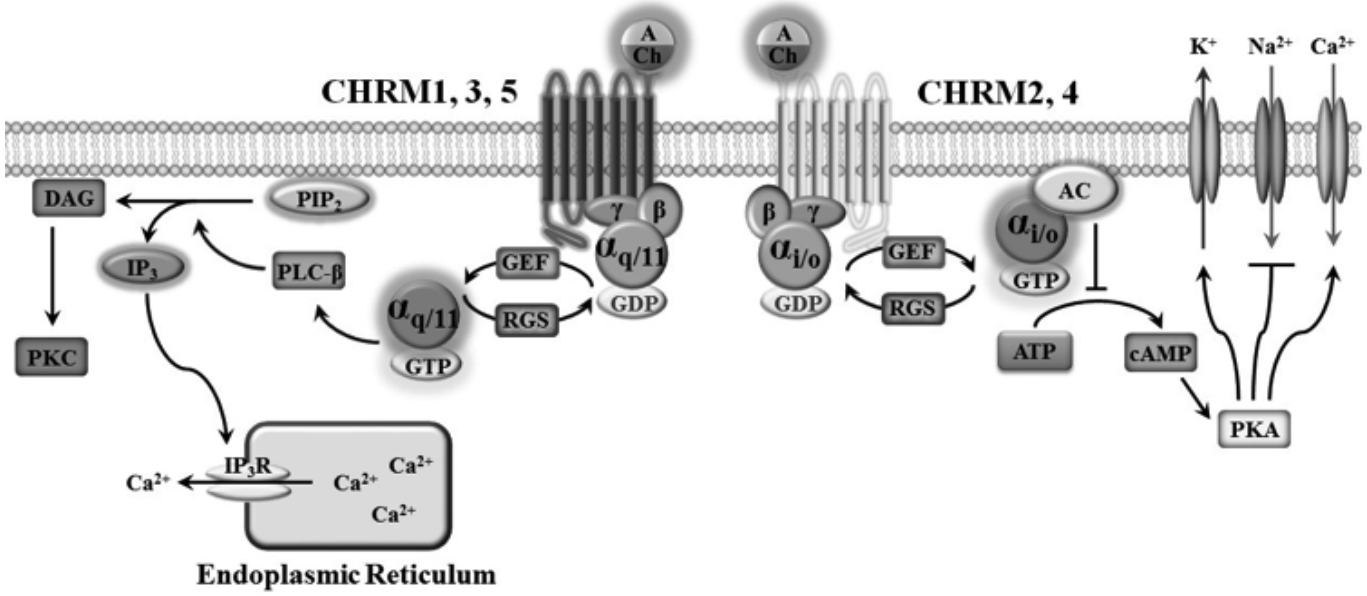

Fig. (1). Schematic showing signal transduction pathways regulated by CHRM1, CHRM3 and CHRM5 coupled to $G_{q/11}$ protein and CHRM2 and CHRM4 coupled to $G_{i/o}$ proteins. AC, adenylate cyclase; ATP, adenosine 5'-triphosphate; cAMP, cyclic adenosine monophosphate; DAG, diacylglycerol; GAP, GTPase-accelerating protein; GDP, guanosine diphosphate; GEF, guanine nucleotide exchange factor; GTP, guanosine triphosphate; IP_3, inositol 1,4,5-trisphosphate; IP_3R, inositol 1,4,5-trisphosphate receptors; PKA, protein kinase A; PKC, protein kinase C; PIP_2, phosphatidylinositol 4,5-bisphosphate; PLC-β, phospholipase C-β; RGS, regulator of G-protein signalling.

Shaw reported that among 16 people who had been exposed to organophosphorus insecticides for up to 10 years; 7 had depression and 5 had schizophrenia with impaired memory, paranoia or/and auditory hallucinations [45, 46]. It was later reported that the infusion of the cholinesterase inhibitor, physostigmine, exacerbated the depressive symptoms in patients with BPD [47, 48]. In response to these data, coupled with observations that cholinergic stimulation can inhibit adrenergic-stimulated, arousal behaviour [49], Janowsky and colleagues hypothesized that an imbalance between central cholinergic and adrenergic neurotransmitter activity, released from cholinergic nerves in the parasympathetic nervous system and adrenergic nerves in the sympathetic nervous system respectively, could induce mania and depression [16]. Specifically, their hypothesis suggested that a hypocholinergic-hyperadrenegic state could cause mania, whereas a hypercholinergic-hypoadrenegic state could contribute to the symptoms of depression.

Further supporting this hypothesis, activation of the cholinergic system by physostigmine induces hyperactivity of the hypothalamic-pituitary-adrenal axis (HPA or HTPA axis) in premenopausal female, but not male, patients with depression [50]. The HPA axis is regulated by the release of the hypothalamic peptide corticotrophin releasing hormone (CRH), suggesting cholinergic super-sensitivity might be involved in exacerbating the depressive symptoms *via* the HPA axis activation. Moreover, the cholinergic system in the brain stem influences the activation of rapid eye movement sleep (REM) and the non-REM sleep cycle [51]. It is therefore significant that the sensitivity of REM sleep response to cholinergic agonists such as RS86, arecoline and physostigmine is increased in patients with major depression [51-53]. These effects contrast those of scopolamine, a muscarinic antagonist, which decreases REM sleep and increases REM sleep latency [54, 55] in patients with depression [56], suggesting the REM variables are regulated by cholinergic tone in depression.

Animal Models of Mood Disorders

The evidence from human pharmacological studies suggesting the cholinergic system is involved in mood disorders is further supported by animal studies that investigated the psychopharmacological effects of antidepressants on the central cholinergic nervous system. The Flinders Sensitive Line (FSL) rat has a behavioural phenotype thought to model some aspects of depression in that it shows a loss of appetite, increases in REM sleep, learning difficulties and immune abnormalities but it does not mimic all biochemical features of depression, such as the noradrenergic, HPA axis or γ-aminobutyric acid (GABA)ergic components [64]. Significantly, this strain of rat was developed by the selective breeding of Sprague-Dawley (SD) rats for high sensitivity to anticholinesterase [57-59]. Thus, it is significant that there is no difference in the CNS binding densities of the broadly selective CHRM radioligand [^{3}H]quinuclidinyl benzilate (QNB) in FSL rats compared to their control line [60], despite them being more sensitive to muscarinic agents in terms of their behavioural and physiological effects, including hypothermia, a decrease in locomotor activity, and suppression of operant response for water reward [61], compared to FRL rats [57, 62]. Thus, the FSL rats may be of particular interest for investigating the mechanisms of the cholinergic system as an animal model of depression.

Another potential model of depression was developed by selectively breeding CD-1® mice for either a high immobility score (>115 seconds) for Helpless (HL) or a low immobility score (<35 seconds) for non-Helpless (NHL) phenotypes using the tail suspension test (TST), an established behavioural model used for identifying the potential antidepressant properties [63, 64]. Compared to NHL mice, HL mice show impairments in behavioural tests such as the forced-swim test (FST) and locomotor activity as well as increased REM sleep and a lighter slow wave sleep (SWS: deep or restorative sleep of the non-REM type) [64]. Moreover, acute treatment with tricyclic antidepressants (TCAs), including imipramine and desipramine, which has a direct anticholinergic effect [65], reduced the immobility in the TST in the HL mice [64]. However, of importance to this review, the muscarinic agonist arecoline, reduced REM sleep latency in the HL mice [66], suggesting cholinergic tone plays an important role in the onset of REM sleep.

In conclusion, studies on FSL rat and the HL mouse are useful for behavioural, neurochemical and pharmacological research as models of depressive disorder that have cholinergic super-sensitivity, consistent with the notion that super-sensitive muscarinic cholinergic receptors are an etiologic factor in mood disorders.

Genetic Studies on Human CHRM2 in Mood Disorders

Evidence implicating the CHRMs in the cholinergic dysfunction seen in mood disorders comes from genetic association studies, which point to a role for CHRM2 in BPD and MDD (Table **1**). One study reported variation at the A1890T (rs8191992) single nucleotide polymorphism (SNP) in the 3' untranslated region (3'UTR) of the *CHRM2* gene was associated with MDD in women, but not in men, with a significant increase in the frequency of homozygosity at the A1890T SNP reported in women with MDD compared to healthy controls [67]. In addition, modest associations have been found between variation in the *CHRM2* gene sequence and populations of European-Americans and African-Americans with affective disorders, where the T-C-A-G-T-T/C-T-C-A-A-A diplotype at rs978437-rs1455858-rs1824024-rs324640-rs324650-rs6962027 was associated with an increased risk of affective disorders [68]. Furthermore, a combination of SNPs (T-T-T haplotype, at rs1824024-rs2061174-rs324650, which has a frequency of over 40% in people with alcohol dependence and major depressive syndrome) at the 5' end of the *CHRM2* gene was found to be associated with alcoholism and MDD, based on the collaborative study on the genetics of alcoholism (COGA) [15]. However, Cohen-Woods and colleagues failed to confirm the association with this haplotype at the SNPs of *CHRM2* gene and MDD reported by Wang *et al*. [15] in a cohort of clinical cases with MDD from the Depression Case Control (DeCC) study [16]. Although genetic studies associated with BPD have identified several candidate chromosome regions [69], similar studies failed to

Table 1. Summary of genetic studies of human CHRMs in mood disorders.

Study	Authors	Types of Experiment	Sample Size	Effect
Genetic	Comings *et al*., 2002 [17]	SNPs (*CHRM2*)	Healthy controls (n = 582; 278 men and 304 women) MDD (n = 178; 52 men and 126 women)	Association between homozygosity at the A1890T (rs8191992) of *CHRM2* and women with MDD
	Wang *et al*., 2004 [109]	SNPs (*CHRM2*)	Alcohol dependence (n = 1,034), MDD syndrome (n = 733) Alcohol dependence or MDD syndrome (n = 1,346) Alcohol dependence and MDD syndrome (n = 461)	Association between SNPs (T-T-T haplotype, at rs1824024-rs2061174-rs324650) of *CHRM2* and alcoholism and MDD
	Luo *et al*., 2005 [63]	SNPs (*CHRM2*)	Healthy controls: European-Americans (n = 287) and African-Americans (n = 46) Affected disorder: European-Americans (n = 382) and African-Americans (n = 156)	Association between diplotype (T-C-A-G-T-T/C-T-C-A-A-A) and an increased risk of affective disorders
	Shi *et al*., 2007 [100]	SNPs	Healthy controls (n = 357) BPD-I (n = 156) BPD-II (n = 30) Schizoaffective disorder bipolar type (n = 15)	No association between SNPs of *CHRMs* and BPD
	Cohen-Woods *et al*., 2009 [16]	SNPs (*CHRM2*)	Healthy controls (n = 1,624; 698 men, 922 women and 4 sex unknown) MDD (n = 1,420; 435 men, 983 women and 2 sex unknown)	No association of SNPs of *CHRM2* in MDD

find an association between BPD and a total of 93 SNPs in 19 cholinergic genes, including all CHRMs [70].

Neuroimaging and Post-mortem Studies of Human CHRM2 in Mood Disorders

Neuroimaging techniques now provide opportunities to study receptor status in the human CNS and how they may be affected in psychiatric disorders [71]. Such an approach, using positron emission tomography and a selective CHRM2 agonist radioligand, 3-(3-(3-fluoroproply) thio)-1,2,5-thiadiazol-4-yl] -1,2,5,6-tetrahydro-1-methylpyridine labelled with F-18 ([^{18}F]FP-TZTP), reported the distribution volume (DV; DV = K_1/K_2, where K1 is the rate of delivery of [^{18}F]FP-TZTP or influx constant from plasma to tissue and K_2 is the rate of clearance) of [^{18}F]FP-TZTP was significantly lower in the anterior cingulate cortex (ACC) from patients with BPD, but not MDD, during depressive episodes. Apparent low levels of binding were also seen in the dorsal and posterior cingulate cortices, the orbital cortex and the visual cortex, but not the amygdala, the hippocampus and the ventral striatum, from patients with BPD but these differences failed to remain significant following statistical correction of the data for multiple comparisons, suggesting changes in CHRM2 may be more widespread in the CNS rather than just being present in the ACC [13]. The smaller distribution volume in the BPD group compared to controls could be due to either a decrease in CHRM2 receptor density, a change in affinity for the ligand or an increase in endogenous ACh levels, as [^{18}F]FP-TZTP binding is sensitive to changes in endogenous ACh concentration due to direct competition between the two ligands [72]. Irrespective of the cause, it is significant that in a follow-up to the study reporting lower density of the PET ligand [^{18}F]FP-TZTP in the ACC from patients with BPD [13], Cannon and colleagues reported that BPD subjects with T-T genotype at the rs324650 SNP showed significantly lower CHRM2 distribution volume compared to both other BPD subjects with A-T or A-A genotype and healthy controls who were also T-homozygotes [73], suggesting a genetic predisposition to lower levels of [^{18}F]FP-TZTP binding. rs324650 lays within the intronic region between exons 5 and 6 of the *CHRM2* and variation within the SNP does not alter the protein's sequence. The reasons why variation at this SNP affects CHRM2 levels in BPD is not clear. However, the *CHRM2* gene is reported to contain regulatory elements within its intronic regions and this SNP may affect gene regulation or splice variation [74, 75]. Alternatively it is possible that the rs324650 was in high linkage disequilibrium with another SNP that affects CHRM2 levels.

In addition, radioligand binding studies using selective muscarinic antagonists have commonly been used to examine the density and regional distribution of muscarinic receptors in human post-mortem brain tissue [14, 33, 76] (Table **2**). Contrasting findings from PET studies, a post-mortem study has reported no change in the density of the CHRM2/CHRM4 selective radioligand [^{3}H]AF-DX 384 in post-mortem ACC (BA 24a and b, including small amounts of BA 32 and/or 33) from people with BPD [77]. The differences between the post-mortem and the PET study findings may be explained by the different genetic backgrounds of their cohorts. However, the PET and post-mortem studies estimated of CHRM2 levels from the radioligand occupation of the receptor's functional vs non-functional binding sites, respectively. Therefore, the lower [^{18}F]FP-TZTP binding levels reported in the PET study could also reflect increased occupation of the CHRM2 functional binding sites by higher levels of exogenous ACh

in the ACC of people with BPD [13]. However, we have reported that [^{3}H]AF-DX 384 binding is significantly lower in the dorsolateral prefrontal cortex (DLPFC; Brodmann's area (BA) 46) from people with BPD and MDD when compared to control subjects [14], supporting the posit that lower [^{18}F]FP-TZTP binding levels in the ACC may reflect lower CHRM2 levels in this region in people with BPD. Our study also reported a lower binding density of the CHRM1/CHRM3 selective radioligand [^{3}H]4-DAMP in BA 10 from subjects with BPD [14]. However, a more recent study by our group showed that under the assay condition used in the earlier study, [^{3}H]4-DAMP was not strongly selective for CHRM3 [33]. Moreover, using a modified assay that used pirenzepine to block CHRM1 and hence increase selectivity for CHRM3, we have now reported that [^{3}H]4-DAMP binding, CHRM3 protein and CHRM3 mRNA are not altered in BA 10 in either BPD or MDD [52] (Table **2**). Furthermore, no change in the density of [^{3}H]pirenzepine binding was observed in either BPD or MDD in any brain region examined [14] or in BA 10 [33], suggesting CHRM1/CHRM4 densities are not altered in either BPD or MDD, supporting an earlier study which showed no change in [^{3}H]pirenzepine density in the ACC in BPD or MDD [78]. Taken together, these data suggest that regionally specific decreases in the level of cortical CHRM2, but not CHRM1 and CHRM3, could be involved in the pathophysiology of mood disorders.

Physiological Consequences of the Decrease in CHRM2

Our understanding of the consequences of a loss of CHRM2 in the CNS comes predominantly from the *Chrm2* KO mice which suggest the receptor plays vital roles in behavioural flexibility [98], working memory [79-81] and hippocampal plasticity [80]. *Chrm2* KO animals showed an increase in ACh levels in the hippocampus and an impaired performance in the passive avoidance test, which was used to evaluate learning and memory in an aversive stimulus (a mild foot shock)-induced passive-avoidance response, suggesting high levels of ACh by the lack of CHRM2 autoreceptors may contribute to some of cognitive deficits [81]. *Chrm2* KO mice showed a significant increase in latencies of the escape response in the Barnes circular maze, during the first 5 days of testing, compared to wildtype (WT) mice. In addition, the correct arm choices for food were decreased in *Chrm2* KO mice in T-maze delayed alternation [80], suggesting *Chrm2* KO mice show deficits in learning and memory. Cognitive impairment, such as learning and memory, has been widely found in patients with MDD [82] and are a common symptom of BPD [83-85].

The PET study [13] suggests lower levels of CHRM2 or a decreased availability of that receptor are present in the ACC, whilst one of 2 post-mortem studies report a lower [^{3}H]AF-DX 384 binding density in the DFPLC from people with mood disorders [14]. These results could indicate

Table 2. Summary of radioligand binding, mRNA and protein studies of human CHRMs in mood disorders.

Study	Authors	Types of Experiment	Region	Effect
Radioligand binding	Zavitsanou *et al*., 2004 [115]	[^{3}H]pirenzepine binding (CHRM1 ≥ 4)	Anterior cingulate cortex (BA 24a and 24b, together with a small contribution from BA 32 and/or 33)	No change
	Zavitsanou *et al*., 2005 [116]	[^{3}H]AF-DX 384 binding (CHRM2 ≥ 4)	Anterior cingulate cortex (BA 24a and 24b, together with a small contribution from BA 32 and/or 33)	No change
	Gibbons *et al*., 2009 [39]	[^{3}H]pirenzepine binding (CHRM1 >> 4)	Rostral prefrontal cortex (BA 10)	No change
			Parietal cortex (BA 40)	No change
			Dorsolateral prefrontal cortex (BA 46)	No change
		[^{3}H]4-DAMP binding (CHRM3 > 1)	Rostral prefrontal cortex (BA 10)	Lower levels in BPD
			Parietal cortex (BA 40)	No change
			Dorsolateral prefrontal cortex (BA 46)	No change
		[^{3}H]AF-DX 384 binding (CHRM2 ≥ 4)	Rostral prefrontal cortex (BA 10)	No change
			Parietal cortex (BA 40)	No change
			Dorsolateral prefrontal cortex (BA 46)	Lower levels in BPD and MDD
	Jeon *et al*., 2013 [52]	[^{3}H]pirenzepine binding (CHRM1 >> 4)	Rostral prefrontal cortex (BA 10)	No change
		[^{3}H]4-DAMP binding (CHRM3 >> 1)	Rostral prefrontal cortex (BA 10)	No change
mRNA	Jeon *et al*., 2013 [52]	qPCR (CHRM3)	Rostral prefrontal cortex (BA 10)	No change
Protein	Jeon *et al*., 2013 [52]	Western blot (CHRM3)	Rostral prefrontal cortex (BA 10)	No change

changes in the autoreceptor function played by CHRM2 in the human cortex. CHRM2 receptors, acting as autoreceptors, inhibit further release of ACh from the presynaptic neurons into the synaptic cleft in the cerebral cortex and regulate the molecular mechanisms of postsynaptic cholinergic receptors [41]. Thus, a decrease in CHRM2 distribution volume may reflect an increase in endogenous ACh levels in the ACC of patients with BPD [13]. This data is supported by an animal study showing that CHRM2 antagonist gallamine increased extracellular ACh levels and, behaviourally, reduced swimming time in the Porsolt forced swim test [86].

The ACC region is part of the limbic system and is involved in mood changes [87] and in the modulation of emotion [88], whilst the DFPLC is known to play key roles in cognitive processes, including attention, working memory, learning and decision making [89]. These functions could be affected by a loss of CHRM2 in that CNS region. Therefore, lower levels of CHRM2 may contribute to the pathogenesis of the cognitive deficits and mood change associated with MDD and BPD.

NEUROPSYCHOPHARMACOLOGICAL STUDIES IN MOOD DISORDERS

The antidepressant effects of biperiden, which is more selective for CHRM1 as an anticholinergic agent [31, 112], showed a significant improvement in depressive patients in an open-label study [54] and in an acute placebo controlled study [4]. However, in a randomized, double-blind, 6-week clinical study, these results did not confirm that biperiden has antidepressant effects compared to the placebo and glycopyrrolate, which is a peripherally effective anticholinergic agent [40].

The evidence supporting the involvement of CHRM2 in mood disorders is pertinent in light of recent reports that scopolamine reduces symptoms of depression in people with either MDD or BPD. Early clinical studies initially failed to show an antidepressant effect of scopolamine. In these studies, elderly patients with depression were treated with different dosages (0.1, 0.25 and 0.5 mg) of scopolamine [76]. 0.5 mg caused significant cognitive impairments in learning, including semantic memory, vigilance, attention, and continuous performance, as well as behavioural changes, including drowsiness, motor restlessness, disjointed speech and anxiety, but the depressive symptoms were not significantly altered an any dose of scopolamine. These data indicated that the symptoms of depression were not strongly influenced by the omnibus activity of CHRMs in human CNS. However, scopolamine (0.4 mg) was observed to reduce REM sleep and prolong REM latency in patients with depression [41], suggesting the REM variables are regulated by cholinergic tone in depression.

More recently, a proof of principle study showed that intravenous infusions of a much lower dose (0.004 mg/kg) of scopolamine significantly reduced scores on the Montgomery–Åsberg Depression Rating Scale (MADRS [75]) and the Hamilton Anxiety Rating Scale (HARS [45, 64]) in 8 depressed patients (5 with MDD and 3 with BPD) compared to the placebo group within 3 to 5 days after infusions [36]. Subsequently, 18 depressed patients with MDD (n = 9) and BPD (n = 9) received intravenous scopolamine (0.004 mg/kg) in a double-blind, placebo-controlled trial. Overall, 61.1% of patients achieved remission (11 of 18; HADRS final score $\leq$ 10), and 55.6% of patients had a full response (10 of 18; HADRS reduction $\geq$ 50%). However, scopolamine treatment did not result in an improvement in scores on the Young Mania Rating Scale (YMRS [98]) for patients with BPD compared to their baseline score, suggesting scopolamine has an antidepressant effect only [11]. More recently, these authors and colleagues confirmed that scopolamine is effective in the treatment of new patients with MDD (n = 23; 22 were included in analyses) [12], and they found that women (n = 31) are 33% more sensitive than men (n = 19) to the antidepressant effects [99]. Thus, more recent studies of the clinical effects of cholinergic antagonists provide considerable support for the cholinergic imbalance hypothesis of depression and suggest the earlier studies failed because the doses of scopolamine used were not titred to weight.

POTENTIAL PHARMACEUTICAL DEVELOPMENT FOR MOOD DISORDERS

As reviewed above, the CHRM2 as an autoreceptor in the cerebral cortex is involved in the pathophysiology of mood disorders. Thus, if the lower levels of CHRM2 in mood disorders [13, 14] are consistent with lower receptor activity, it would be expected there would be an increased release of ACh into the synaptic cleft, resulting in an overall hypercholinergic state. Scopolamine might inhibit the resulting increase in post-synaptic CHRM signalling, which may explain its mechanism of therapeutic action. This notion is supported by animal studies using gallamine, a CHRM2 antagonist, which show an increase in extracellular ACh levels in the nucleus accumbens [86]. However, scopolamine caused an increase in swimming time [86], suggesting an overall effect of that drug at certain doses might be to create a relatively hypercholinergic state due to an increase in the release of ACh. By contrast, the CHRMs agonist arecoline decreased swimming time in the forced swim test and showed a reduction in open-field locomotion activity [86, 100], suggesting that cholinergic activation causes depressive-like behaviour. Hence, these data suggest specifically targeting the CHRM2 with an antagonist may be efficacious and produce rapid and robust effects in the treatment of mood disorders.

This review provides evidence in support of the hypothesis that there is an imbalance between the central cholinergic and adrenergic neurotransmitter activity in mood disorders [16]. Data is also presented that argues that drugs targeting specific CHRM2 which regulate the release of ACh could be used for the treatment of mood disorders. Despite their potential therapeutic benefits for mood disorders, the clinical use of agonists or antagonists, which bind to the orthosteric binding site of receptors, could theoretically not be very effective. For example, orthosteric agonists can cause receptor desensitization/internalization and down-regulation [101], resulting in short-lived efficacy. Another disadvantage is that full antagonists, such as a scopolamine, with poor selectivity could influence both presynaptic CHRM2 and other postsynaptic cholinergic receptor subtypes, suggesting that it may not reach maximum therapeutic effect. In order to overcome these disadvantages, an alternative approach is the development of allosteric modulators, which bind to an allosteric site on the receptor [102], either enhance or reduce

the effect of the orthosteric ligand and show a great selectivity for the specific CHRM2 subtype [35, 103]. The allosteric modulator has shown no influence on binding of orthosteric agonist on the receptor and does not appear to have the problem of receptor desensitization and down-regulation [104] seen with orthosteric agonists. An allosteric modulator targeting presynaptic CHRM2 has different mechanisms of action in comparison with orthosteric agonists [35, 104] and produces synergistic effects of the endogenous ACh by enhancing the affinity of ACh for the orthosteric binding site on CHRM2. Thus, it may be possible to develop a positive allosteric modulator selectively targeting CHRM2 to effectively treat patients suffering from mood disorders.

Early studies using cholinesterase inhibitors, such as organophosphorus insecticides and physostigmine, which increase levels of ACh in the synaptic cleft, caused depressive symptoms in healthy people and improved manic symptoms in patients with mania [44, 47]. Moreover, the treatment with cholinesterase inhibitors plus a Neuro Psychological Training (TNP) significantly reduced scores on the Geriatric Depression Scale (GDS [105]), compared to their baseline score, resulting in improvement of depressive symptoms and showed significant improvement of cognition in patient with mild cognitive impairment [106]. Results from animal studies of *Chrm2* KO showed impaired performance in the passive avoidance test, suggesting CHRM2 is involved with cognitive processes [81]. A CHRM2 antagonist increased extracellular ACh levels in the nucleus accumbens [86], resulting in compensatory higher levels of acetylcholinesterase [107]. An association between lower levels of CHRM2 and cholinesterase functions in mood disorders may contribute to some of cognitive deficits associated with mood disorders. Given that cognitive impairment is widely reported in patients with MDD [82] and a common symptom of BPD [83-85], although it is still unclear about these processes between the cholinesterase inhibitors and the selective modulation of CHRM2 because of the possibility of counteraction each other, drug development targeting them would be an important approach to provide enhancement of cognitive function in novel therapeutic mechanism for the treatment of mood disorders.

Finally, some conventional tricyclic antidepressants (TCAs), such as imipramine, which primarily inhibits norepinephrine (noradrenaline) re-uptake [108] but has additional pharmacological activities, have been associated with direct anticholinergic effects [65]. However, the mechanism underpinning this action of TCAs is not fully understood. Although TCAs have adverse side effects due to the antimuscarinic actions [109-111], it can be suggested that these drugs could be effective in treating patients with depression through the potent antagonism of muscarinic receptors, as seen with scopolamine [11, 99].

CONCLUSION

Data from genetic, neuroimaging and radioligand binding studies support the involvement of CHRM2 in the pathophysiology of MDD and BPD. However, investigating the factors that regulate the expression of CHRM2 remains to be clearly elucidated in the aetiology of these disorders. Thus, further studies are needed to fully understand the regulation of CHRM2 expression in the pathophysiology of mood disorders. First of all, it is important that these data would be replicated with a large number of samples to provide more reliable conclusion. Future studies should investigate an interaction between the SNPs of *CHRM2* gene and environmental and biochemical factors or a combination of these factors associated with mood disorders to identify the regulatory mechanism underlying the effects of the allele on CHRM2 expression levels. Lastly, the association with CHRM2 expression and clinical outcomes obtained from biological, psychological and cognitive measures needs to be further confirmed in the future study.

In developing drugs to target the CHRMs to treat mood disorders, it must be recognised that this receptor is abundant in CNS and the peripheral nervous system (PNS), as well as being present in non-neuronal peripheral tissues such as smooth muscle [112] and heart [113]. In order to avoid the adverse effects associated with targeting the peripheral receptors, drugs targeting specific CHRM2 would need to be delivered to the CNS, but not the PNS [114, 115]. The main problem in the drug delivery into the CNS is that the blood-brain barrier (BBB) as a highly selective permeability barrier allows only small-molecule drugs to be carried into the CNS [116]. Thus, to overcome these properties of the BBB in the drug delivery into the CNS, a promising approach for drug delivery into the CNS is the use of invasive techniques, which are to penetrate the BBB by using surgical methods, BBB disruption and direct injection into the CNS, and non-invasive methods, which are to penetrate or to bypass the BBB by using carriers, such as liposomes, nanoparticles, and polymeric micells [114, 115, 117]. These multiple drug delivery systems give promise for effective drug delivery to the CNS, showing potential for safer and more effective treatment for mood disorders. Hence, drugs selectively targeting the central CHRM2 will have fewer of the common side-effects, such as drowsiness, blurred vision and dry mouth, associated with muscarinic receptor antagonists, including scopolamine, and TCAs. The concept of developing selective ligands targeting the CHRM2 provides a new and feasible approach for the specific CNS drug delivery to more effectively treat mood disorders.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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