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Pharmacology of dextromethorphan: Relevance to dextromethorphan/quinidine (Nuedexta®) clinical use

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ABSTRACT

Dextromethorphan (DM) has been used for more than 50 years as an over-the-counter antitussive. Studies have revealed a complex pharmacology of DM with mechanisms beyond blockade of N-methyl-D-aspartate (NMDA) receptors and inhibition of glutamate excitotoxicity, likely contributing to its pharmacological activity and clinical potential.

DM is rapidly metabolized to dextrorphan, which has hampered the exploration of DM therapy separate from its metabolites. Coadministration of DM with a low dose of quinidine inhibits DM metabolism, yields greater bioavailability and enables more specific testing of the therapeutic properties of DM apart from its metabolites. The development of the drug combination DM hydrobromide and quinidine sulfate (DM/Q), with subsequent approval by the US Food and Drug Administration for pseudobulbar affect, led to renewed interest in understanding DM pharmacology.

This review summarizes the interactions of DM with brain receptors and transporters and also considers its metabolic and pharmacokinetic properties. To assess the potential clinical relevance of these interactions, we provide an analysis comparing DM activity from in vitro functional assays with the estimated free drug DM concentrations in the brain following oral DM/Q administration. The findings suggest that DM/Q likely inhibits serotonin and norepinephrine reuptake and also blocks NMDA receptors with rapid kinetics. Use of DM/Q may also antagonize nicotinic acetylcholine receptors, particularly those composed of $\alpha_3\beta_4$ subunits, and cause agonist activity at sigma-1 receptors.

Contents

1. Introduction

Dextromethorphan (DM) has long been used as an over-the-counter cough suppressant, though the molecular mechanism to suppress coughing is not well established (Kamei et al., 1989; Brown et al., 2004; Kim et al., 2009; Canning & Mori, 2010; Young & Smith, 2011). DM has a multi-faceted pharmacology and has interactions with serotonin transporters, noradrenaline (NA) transporters, sigma-1 receptors (Sig1-R), $\alpha3\beta4$ nicotinic acetylcholine receptors, and N-methyl-D-aspartate receptors (NMDARs). These proteins are present in several neurotransmitter systems that are targeted in the treatment of neurological and psychiatric disorders. Although structurally related to opioid agonists, DM does not have relevant activity at opioid receptors (Kachur et al., 1986; Gaginella et al., 1987; Redwine & Trujillo, 2003; Chen et al., 2005). The receptor pharmacology of DM (particularly at NMDARs) has led clinical researchers to explore its therapeutic potential in conditions such as pain (McQuay et al., 1994; Ilkjaer et al., 2000) and epilepsy (Fisher et al., 1990; Kimiskidis et al., 1999), as a neuroprotectant for acute brain injury or stroke (Albers et al., 1992), and for neurodegenerative disorders (Walker & Hunt, 1989; Gredal et al., 1997). However, most of these early clinical trials of DM failed to show therapeutic utility. The lack of therapeutic response may have been influenced by low and variable bioavailability of DM when administered alone because of its rapid first-pass metabolism and subsequent elimination.

In order to increase plasma concentrations and bioavailability in the central nervous system (CNS), DM was combined with quinidine (Q), a potent inhibitor of the cytochrome P450 (CYP) liver enzyme CYP2D6, which is the primary enzyme involved in DM metabolism (Fig. 1). The combined dextromethorphan and quinidine formulation is referred to as DM/Q. The addition of quinidine in the DM/Q combined dosage form causes substantial changes in the circulating amounts of both dextromethorphan and dextrorphan (DX), with the predominant active compound changing from dextrorphan when DM is given alone, as with cough suppressants, to dextromethorphan when given as DM/Q. For example, the ratio of circulating free DX to free DM is 6.4-fold in favor of dextrorphan with DM given alone but is 14-fold in favor of dextromethorphan with DM/Q (see Fig. 2B and further explanation in Section 2, Dextromethorphan metabolism and pharmacokinetics).

Pharmacokinetic studies evaluating this combination demonstrated large increases in DM bioavailability in the presence of quinidine, and this led to renewed interest in clinical trials culminating in the combination of DM hydrobromide and Q sulfate (DM/Q; Nuedexta®) being approved by the US Food and Drug Administration in 2010 and by the European Medicines Agency in 2013 as a treatment for the neurological condition, pseudobulbar affect (PBA). PBA can occur in persons with neurological disorders affecting the brain or with brain injury and is characterized by frequent, involuntary and uncontrollable laughing or crying episodes that can be incongruent with mood or social context.

DM/Q has also shown therapeutic effects in well-controlled clinical trials for diabetic peripheral neuropathic pain (Shaibani et al., 2012) and agitation secondary to Alzheimer's disease (Cummings et al., 2015). In a small pilot study, DM/Q therapy reduced the abnormally large late auditory evoked potential (N400) produced by hearing a familiar name in patients with PBA secondary to multiple sclerosis, consistent with DM/Q effects on neocortical brain activity in these patients (Haiman et al., 2009). A preliminary retrospective case series of patients treated for bipolar depression (Kelly & Lieberman, 2014) and chorea of various etiologies (Ondo, 2012) also suggested this drug combination may have therapeutic effects in these CNS disorders (Lauterbach, 2012). The precise molecular mechanism of action of DM/Q in each of these disparate conditions is unknown, and actions at more than one receptor or transporter might be involved.

This review summarizes the interaction of DM with various CNS receptors and transporters and also considers its rapid first-pass metabolism to dextrorphan (DX). In addition to reviewing findings in the literature, this paper presents an original analysis of the therapeutic relevance of DM receptor interactions based on plasma drug concentrations and predicted cerebrospinal fluid (CSF) concentrations using data from clinical studies of DM/Q.
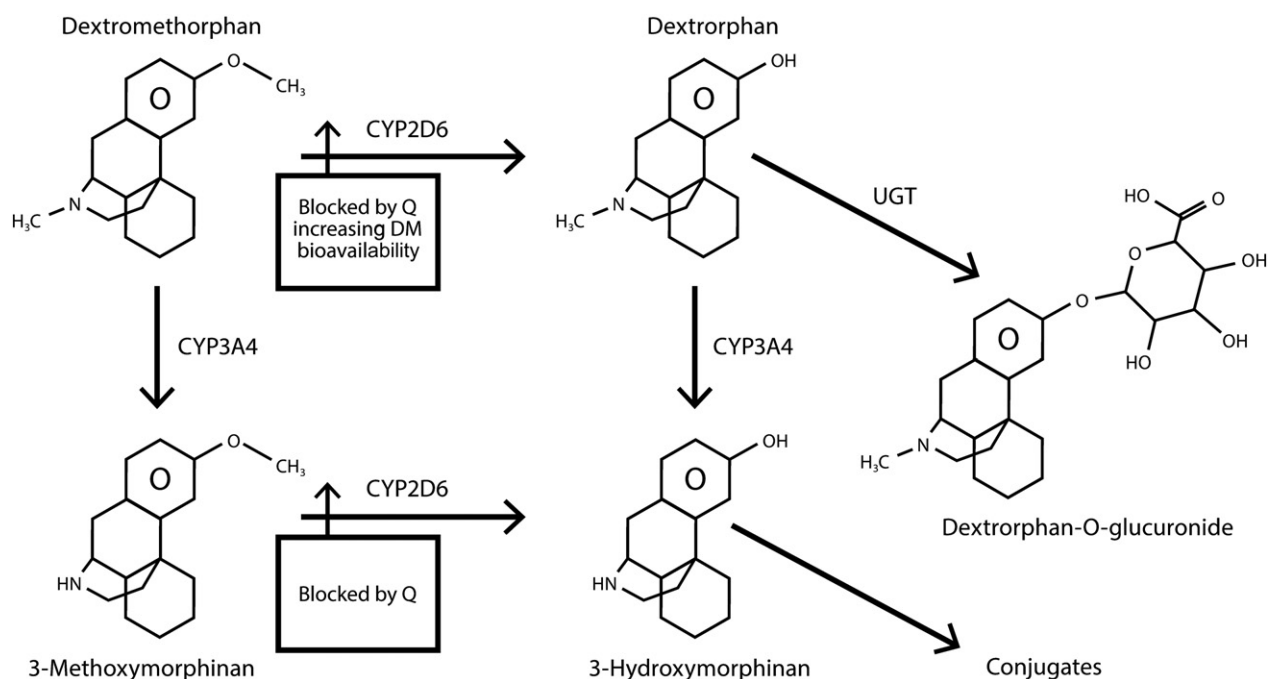
Fig. 1. Dextromethorphan metabolism. Dextromethorphan in extensive metabolizers is rapidly metabolized (mostly in liver) to form dextrorphan. In poor metabolizers and in persons treated with quinidine, this metabolism is mostly blocked. Subsequently, most dextrorphan is rapidly glucuronidated. Demethylation by CYP3A4 to form 3-methoxymorphinan is a relatively minor pathway. Both 3-methoxymorphinan and dextrorphan are further metabolized to 3-hydroxymorphinan. Dextrorphan has significant pharmacological activity, particularly at NMDA receptors. UGT — uridine 5′-diphospho-glucuronosyltransferase; DM – dextromethorphan; Q – quinidine. Figure is adapted with permission from Lutz and Isoherranen (2012).
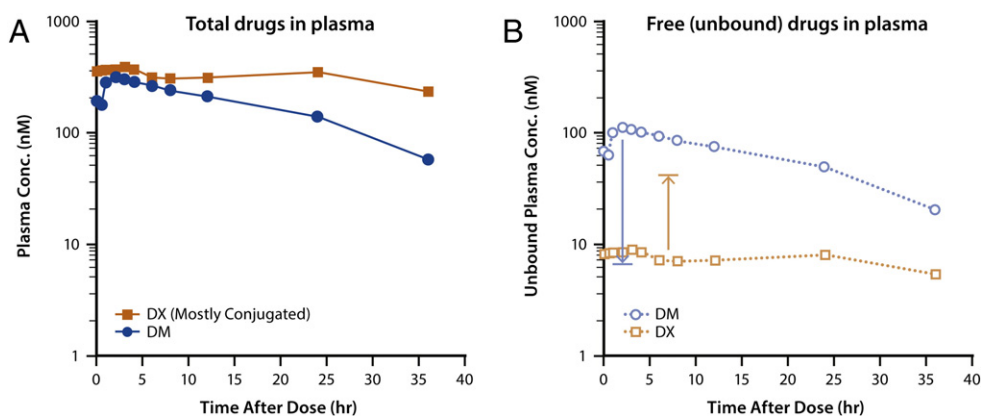
Fig. 2. Dextromethorphan and dextrorphan concentrations in human plasma with DM/Q oral administration. Healthy subjects were given 30 mg dextromethorphan (DM) plus 10 mg quinidine to block DM metabolism daily for 7 days. Total drug in plasma was measured and free concentration in plasma was calculated. **A.** Plasma drug concentrations (DM and dextrorphan, DX) were obtained at various times after dosing (time 0) on day 7. This graph shows total plasma concentrations of DM (protein-bound plus unbound, blue circles) and DX (conjugated plus non-conjugated, orange squares). **B** shows free (unbound) concentrations of DM and DX based on data as measured in part **A**. Free DM (light blue open circles) was calculated considering 65% DM binding to plasma proteins (Avanir Pharmaceuticals, Inc., 2010), and non-glucuronidated free DX (light orange open squares) was calculated assuming covalently glucuronidated DX is 33 to 43-fold greater than non-glucuronidated free DX (Chen et al., 1990; Kazis et al., 1996). Data points are means from 4 subjects from an unpublished Avanir study (13-AVR-134). Similar dosing of 30 mg DM without quinidine resulted in unbound peak DM concentrations that were 25-fold lower (blue arrow) and peak free DX concentrations that were 4-fold greater (orange arrow), so DX represents the major active compound when quinidine is not used, while DM predominates when quinidine is used. Unbound concentrations are assumed to be in equilibrium with brain extracellular fluid. In summary, blocking DM metabolism changed the ratio of free DM to free DX by ~100-fold.

2. Dextromethorphan metabolism and pharmacokinetics

In ~90% of individuals, referred to as extensive metabolizers, DM undergoes rapid and extensive first-pass metabolism to the major O-demethylated metabolite dextrorphan (DX) mediated by CYP2D6 (Fig. 1). As CYP2D6 is a polymorphically expressed enzyme, some individuals lack CYP2D6 activity (poor metabolizers) and others express varying levels of enzyme activity. In a less prominent elimination pathway, DM undergoes *N*-demethylation to 3-methoxymorphinan via CYP3A4/A5. Both DX and 3-methoxymorphinan undergo further demethylation to 3-hydroxymorphinan.

When DM is administered by itself orally (30 mg) to extensive metabolizers, only low plasma levels (unbound plasma concentrations of 2 to 4 nM) are achieved due to rapid and extensive metabolism to DX (Chen et al., 1990; Pope et al., 2004). Once DX is formed, it is glucuronidated by uridine diphosphate-glucuronosyltransferase to form dextrorphan-O-glucuronide, and most DX (~97 to 98%) is present in plasma as the O-glucuronide (Chen et al., 1990; Kazis et al., 1996). Dextrorphan-O-glucuronide is permanently charged and less permeable to the blood–brain barrier than unconjugated DX, and therefore is unlikely to produce significant pharmacological effects in the brain at clinically used doses even though it may be present at higher total (bound and unbound) plasma concentrations than DM. Furthermore, glucuronidated compounds usually are rapidly eliminated by the kidneys (Wu et al., 1995). As unconjugated 3-methoxymorphinan and 3-hydroxymorphinan (3-hydroxymorphinan is also rapidly glucuronidated) are found at concentrations even lower than those of DM in both extensive and poor metabolizers, their pharmacology is not discussed in this review.

2.1. Increasing dextromethorphan exposure by blocking its metabolism

Studies in healthy human volunteers show that coadministration of the CYP2D6 inhibitor, quinidine, decreases the rate of metabolism of DM, thereby increasing its elimination half-life ($t_{1/2}$) from ~4 h (Kazis et al., 1996) to ~13 h (Pope et al., 2004) in volunteers with an extensive metabolizer phenotype, allowing twice-daily administration to maintain consistent DM plasma concentrations. For instance, at the DM/Q FDA-approved dosage for the treatment of PBA (DM 20 mg and quinidine 10 mg, administered twice daily), the maximum plasma concentration (C_{max}) of DM is ~51 ng/mL (66 nM unbound DM) (Pope et al.,

2004; Avanir Pharmaceuticals, Inc., 2010). In contrast, in extensive metabolizers receiving DM 30 mg without quinidine, the C_{max} for DM is only 2.9 ng/mL (3.7 nM unbound) (Pope et al., 2004). These findings and the pharmacokinetic profile for DM following administration of DM/Q are summarized in Fig. 2. It is also important to note that the daily dose of quinidine in DM/Q is much lower than the antiarrhythmic dose of quinidine that is used to block cardiac sodium channels. Use of DM/Q at approved doses (20/10 mg twice daily), results in a maximum quinidine plasma concentration (C_{max}) that is only 1% to 3% of the concentration required for antiarrhythmic efficacy.

Several clinical studies suggest that unchanged DM is responsible for pharmacodynamic effects observed in humans following dosing with DM plus quinidine, despite the slightly higher total plasma concentrations of DX (much of which is conjugated, see Fig. 2). For example, DM plus quinidine significantly reduced the requirement for nonsteroidal anti-inflammatory drugs after knee replacement surgery compared with DM plus placebo (Ehret et al., 2013). Likewise for PBA, DM plus quinidine demonstrated significant efficacy compared to placebo (Panitch et al., 2006; Pioro et al., 2010), and, in a study comparing efficacy of DM/Q to its individual components, the combination was significantly more effective in treating PBA than either DM or Q alone (Brooks et al., 2004).

Limited data on DM and its metabolite concentrations in CSF suggest that DM concentrations in plasma can be used to estimate free DM concentrations present in the extracellular fluid of the brain. Measured CSF concentrations of DM in humans (Steinberg et al., 1996; Lutz & Isoherranen, 2012) were very close to unbound DM in plasma (Fig. 3). The unbound DM concentration is 35% of measured human whole plasma concentrations, as derived from 65% nonspecific binding of DM to plasma proteins (Pope et al., 2004). When DX levels were measured in CSF (Kazis et al., 1996), they were found to be very close to unconjugated DX in plasma. Therefore, for our analysis (detailed in Section 4), we chose unbound DM in plasma and unconjugated DX in plasma as the relevant concentrations for estimating pharmacological actions in the brain.

Studies that measure both DM and DX in the CSF of humans will be needed to determine unbound brain concentrations of DM and DX in patients receiving DM alone or the DM plus Q combination. Nonetheless, in vitro and in vivo data from studies that measured free DX are tabulated in parallel in this review when information is available.
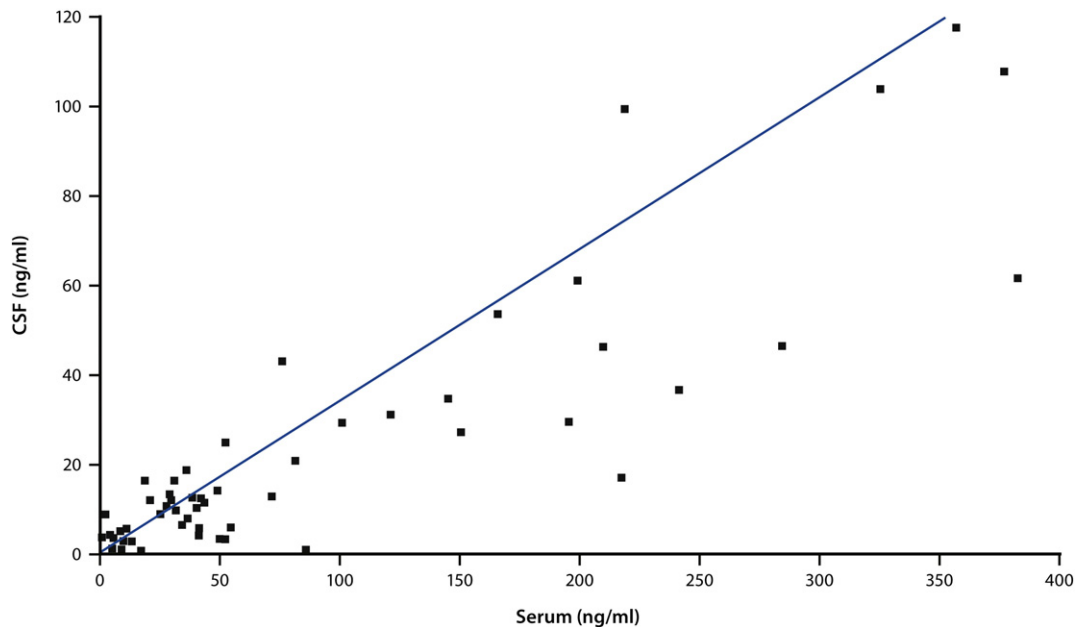
Fig. 3. Concentrations of DM in serum and cerebrospinal fluid (CSF). Serum and CSF concentrations of DM in human patients are correlated ($r = 0.88$, $P < 0.0001$). CSF concentrations are 30% of those in serum (blue line), or very similar to the unbound plasma drug concentration calculated with 65% of DM in plasma bound to plasma proteins, as determined in vitro (Avanir Pharmaceuticals, Inc., 2010). Data presented in the figure are from the Steinberg et al. (1996) study in neurosurgical patients given ascending doses of oral DM to a maximum tolerated dose ranging from 0.8 to 9.6 mg/kg (40 to 480 mg for a 50 kg person). Figure is adapted with permission from Steinberg et al. (1996).

3. Receptor pharmacology of dextromethorphan

3.1. Overview of receptor and transporter interactions

The CNS pharmacology of DM and the likely involvement of NMDARs and Sig1-Rs were reviewed in 1989 by Tortella et al. (1989). More recently, a preclinical pharmacology review provided a tabulated summary of the evidence supporting the neuroprotective effects of DM in various nonclinical models of CNS injury (Werling et al., 2007a). The reader is referred to these reviews for additional information. Here, evidence from an in vitro binding study of DM assessing 26 potential binding sites (Werling et al., 2007b) is discussed, as are previously unpublished data from a second radioligand screening study (Table 1).

These results show that DM competes with radioligand binding at Sig1-Rs and serotonin transporters with high to moderate affinities and with lower affinities to sites on NMDAR and NA transporters (Table 1). DM did not compete with the agonist binding of ^{3}H-epibatidine to the neuronal acetylcholine nicotinic receptor in this study, although functional antagonism has been reported (discussed in Section 3.6). The screening data also show weak potential interactions of DM with adrenergic α1D receptors ($K_i = 830$ nM), rat calcium L-type (benzothiazepine) channels ($K_i = 2800$ nM for enhanced binding), human adrenergic α1A receptors ($K_i = 3000$ nM), and rat sodium (Site 2) channels (57% inhibition with 10,000 nM DM). With weak radioligand binding K_i values of over 1000 nM, these interactions are unlikely to be clinically relevant; however, functional studies are lacking

Table 1
Radioligand binding data with dextromethorphan (DM) and dextrorphan (DX).

Receptor	K_i or % inhibition DM	K_i or % inhibition DX	Radioligand	K_i of radioligand	Reference
5-HT transport	40 nM	484 nM	[^{3}H]paroxetine, 0.2 nM	0.2 nM	Werling et al., 2007b
Sig1-R	150 nM	118 nM	[^{3}H]pentazocine, 2 nM	5 nM	Werling et al., 2007b
NA transport	6000 nM	6200 nM	[^{3}H]nisoxetine, 2 nM	0.7 nM	Pubill et al., 1998
NMDAR	962 nM	148 nM	[^{3}H]MK-801, 0.5 nM	1.6 nM	Jaffe et al., 1989
NMDAR	2120 nM	892 nM	[^{3}H]MK-801, 3 nM	1.6 nM	Werling et al., 2007b
Adrenergic α1D	*830 nM*	*NT*	[^{3}H]prazosin, 0.1 nM	0.1 nM (K_d)	Avanir data on file
Adrenergic α1A	*3000 nM*	*NT*	[^{3}H]prazosin, 0.2 nM	0.15 nM (K_d)	Avanir data on file
Sigma-2	*11,060 nM*	*11,325 nM*	[^{3}H]DTG, 2.5 nM	21 nM	Chou et al., 1999
5-HT$_{1B/D}$	*61% at 1000 nM*	*54% at 1000 nM*	[^{3}H]GR 125,743, 0.3 nM	0.24 nM	Werling et al., 2007b
Adrenergic α-2	*60% at 1000 nM*	*NC*	[^{3}H]yohimbine, 2 nM	1.4 nM	Werling et al., 2007b
Histamine-1	*NC at 1000 nM*	*95% at 1000 nM*	[^{3}H]mepyramine, 2 nM	1.5 nM	Werling et al., 2007b

Abbreviations: 5-HT — serotonin; NA — noradrenaline; NMDAR — N-methyl-D-aspartate glutamate receptor, NT — not tested, NC — no significant competition of radioligand binding (less than 30% inhibition at 1000 nM). Data on adrenergic radioligand binding is from Avanir data on file, obtained from Cerep, Celle l'Evescault, France, July 25, 2012.
Note: Dextromethorphan did not significantly inhibit radioligand binding at 10,000 nM at several other sites (Avanir data on file from Ricerca Biosciences, Taipei, Taiwan, November 2010). These inactive binding sites for dextromethorphan include: adenosine A1, A2A, A3, adrenergic α1B, α2A, β1, β2, bradykinin B1, B2, calcium channel dihydropyridine, calcium channel N-type, cannabinoid CB1, dopamine D1, D2S, D3, D4.2, endothelin ET$_A$, ET$_B$, epidermal growth factor, GABA$_A$ flunitrazepam, GABA$_A$ muscimol, GABA$_{B1A}$, glucocorticoid, glutamate kainate, glutamate NMDAR agonist site, glutamate NMDAR glycine site, strychnine-sensitive glycine, histamine H1, H2, H3, imidazole I2, interleukin IL-1, leukotriene, melatonin MT1, muscarinic M1, M2, M3, neuropeptide Y Y1, Y2, nicotinic α1, opiate μ, δ, κ, phorbol ester, platelet activating factor, potassium channel K$_{ATP}$, potassium channel hERG, prostanoid EP4, purinergic P2X, P2Y, rolipram, serotonin 5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_3$, tachykinin NK1, testosterone, thyroid hormone, dopamine transporter.
Note — Any functional significance of radioligand binding activity at ~1000 nM and greater concentrations and without functional pharmacologic testing is not known and needs confirmation with functional tests (these data are shown in italics in the table). For example, clinically useful serotonin 5-HT$_{1B}$ agonists (sumatriptan, dihydroergotamine) have K_i values for radioligand binding of 3 nM to 440 nM (Buzzi & Moskowitz, 1991), and the selective adrenergic α1D antagonist BMY 7378 has a K_i value for radioligand binding of 0.4 nM (Goetz et al., 1995).

(see additional discussion in Section 4 and also Section 3.7 below, cation channel blocking actions), and therefore, additional studies will be required to determine the relevance of these targets to DM/Q pharmacology.

3.2. Inhibition of serotonin reuptake

Serotonin is a monoamine neurotransmitter involved in the regulation of mood and behavior with 14 known receptors in humans (Donaldson et al., 2013). Serotonin reuptake transporters are a major site of action of many antidepressant drugs. In an in vitro functional study (Codd et al., 1995), DM potently inhibited serotonin reuptake into rat brain synaptosomes with $K_i = 23$ nM (Table 2). Although we could find no experiments directly measuring serotonin concentrations in the brain extracellular space with and without DM treatment (e.g., microdialysis studies), an experiment in rats showed decreased brain concentrations of the serotonin metabolite 5-hydroxyindoleacetic acid after DM doses of 20 to 40 mg/kg i.p., providing indirect evidence of serotonin reuptake inhibition (Ahtee, 1975). Additionally, an in vivo functional study of rats with 6-hydroxydopamine lesions to the substantia nigra (a rat model of Parkinson's disease) showed that DM (45 mg/kg i.p.) partially reversed levodopa-induced abnormal involuntary movements, an effect thought to be mediated, at least in part, by serotonin 5-HT$_{1A}$ autoreceptor stimulation (Paquette et al., 2012). This effect was reversed by a selective serotonin 5-HT$_{1A}$ antagonist and is consistent with DM acting indirectly on 5-HT$_{1A}$ receptors via inhibition of serotonin reuptake. Furthermore, in a model of serotonin syndrome in rabbits, Sinclair (1973) showed that DM (5 mg/kg i.v.) increased body temperature by rapidly enhancing the action of the monoamine oxidase inhibitors phenelzine or nialamide, effects attributed to serotonin reuptake inhibition of DM. Similar findings have been observed in humans when DM (without quinidine) was co-administered with monoamine oxidase inhibitors (Rivers & Horner, 1970; Sovner & Wolfe, 1988), consistent with DM or its metabolites increasing brain serotonin concentrations via serotonin reuptake inhibition.

3.3. Agonist action at sigma-1 receptor sites

The Sig1-R gene, SIGMAR1, is highly conserved across species (Hanner et al., 1996; Kekuda et al., 1996; Pan et al., 1998; Seth et al., 1998) and encodes for a transmembrane protein located in the endoplasmic reticulum (ER) of neurons and other cells (Hayashi, 2015). It exhibits no homology to other mammalian proteins (Hanner et al., 1996), representing a unique structural class of protein that is distinct from G-protein coupled receptors, ligand-gated ion channels and neurotransmitter transporters. Contrasting with the common configuration of neurotransmitter receptors, the Sig1-R binding site is initially located in the inner or luminal surface of the ER membrane (Hayashi, 2015). The Sig1-R functions as a ligand-activated chaperone and can modulate the activity of classical neurotransmitter systems and signaling cascades through direct or indirect interactions with ion channels, receptors, and other cellular proteins and components (Hayashi & Su, 2007; Su et al., 2010; Hayashi, 2015). In the brain,

Sig1-Rs are found in areas such as the cerebellum, brainstem, neocortex, striatum, and hippocampus (Gundlach et al., 1986; McLean & Weber, 1988; Bouchard & Quirion, 1997).

The interaction between DM and Sig1-Rs in the brain was first described in the 1980s. High affinity ligand binding sites for DM were identified in the rodent brain and were subsequently characterized as recognition sites for other Sig1-R ligands (Canoll et al., 1989; Klein & Musacchio, 1989; Musacchio et al., 1989). Using more selective ligands that have since been developed, DM has been confirmed to bind to Sig1-Rs, but not sigma-2 receptors, with significant affinity (Shin et al., 2007; Werling et al., 2007a; Fishback et al., 2012). In the presence of 400 nM DM, radioligand binding of the high-affinity Sig1-R agonist [^{3}H](+)-pentazocine occurred with reductions in both K_d and B_{max}, suggesting complex interactions with Sig1-Rs which may involve competitive as well as non-competitive binding (Nguyen et al., 2014).

A number of studies indicate that DM acts, at least in part, as an agonist at Sig1-Rs. For example, selective Sig1-R antagonists can mitigate the effects of DM in various preclinical models. The hallmark antitussive effect of DM can be attenuated by the Sig1-R antagonist BD1047 in a guinea pig citric-acid cough model (Brown et al., 2004). The previously reported anticonvulsant and neuroprotective effects of DM also are reduced by Sig1-R antagonists such as BD1047 (Kim et al., 2003; Shin et al., 2005, 2007). Also, in rat neocortical brain slices in vitro, DM prevented spreading depression, and this was reversed with Sig1-R antagonists in this experiment (Anderson & Andrew, 2002). Finally, DM elicited antidepressant-like effects in the mouse forced swim and tail suspension tests, which were attenuated by the Sig1-R antagonist BD1063 or the AMPA receptor antagonist NBQX (Nguyen et al., 2014; Nguyen & Matsumoto, 2015).

Other Sig1-R agonists elicit antitussive effects (Brown et al., 2004), convey neuroprotective actions (Nguyen et al., 2015; Ruscher and Wieloch, 2015), and also produce antidepressant effects in animal models (Bermack & Debonnel, 2005; Fishback et al., 2010; Hashimoto, 2015). In contrast, knockdown or knockout of Sig1-Rs can promote cell death and damage (Wang & Duncan, 2006; Hayashi & Su, 2007; Ha et al., 2011; Mavlyutov et al., 2011; Mori et al., 2012; Vollrath et al., 2014; Bernard-Marissal et al., 2015) or a depressive-like behavioral phenotype (Sabino et al., 2009; Chevallier et al., 2011; Sha et al., 2015).

Some effects of Sig1-R agonists have been reported to occur through the modulation of glutamatergic mechanisms (Martina et al., 2007; Balasuriya et al., 2013; Pabba et al., 2014; Nguyen & Matsumoto, 2015) and other well established targets that become engaged under pathological conditions (Bermack & Debonnel, 2005; Maurice & Su, 2009; Nguyen et al., 2014; Hashimoto, 2015).

Specific cellular mechanisms through which Sig1-Rs may mediate the effects of DM have not yet been determined. Systematic studies are still needed to assess the manner and extent to which Sig1-Rs may promote therapeutic actions of DM.

3.4. Inhibition of noradrenaline reuptake

DM has been reported to inhibit [^{3}H]-NA uptake into rat brain synaptosomes in vitro with a K_i value of 240 nM (Codd et al., 1995). A

Table 2
In vitro functional pharmacology data for dextromethorphan and its metabolite dextrorphan.

Test	Tissue source	Test method	DM (nM)	DX (nM)	References
5-HT reuptake inhibition	Rat brain synaptosomes	[^{3}H] 5-HT uptake (K_i)	23 (K_i)	401 (K_i)	Codd et al., 1995
Sig1-R agonist activity (spreading depression)	Rat neocortical brain slice	Neuroimaging and electrophysiological recordings	10,000 nM blocked spreading depression	Not available	Chou et al., 1999
Norepinephrine reuptake inhibition	Rat brain synaptosomes	[^{3}H]NA uptake (K_i)	240 (K_i)	340 (K_i)	Codd et al., 1995
NMDAR ion channel (uncompetitive antagonism)	Mouse neocortex cultured neurons	Voltage clamp (IC$_{50}$)	550 (IC$_{50}$)	72 (IC$_{50}$)	Trube & Netzer, 1994
Nicotinic α3β4 antagonism	Xenopus oocytes	Voltage clamp (IC$_{50}$)	700 (IC$_{50}$)	1300 (IC$_{50}$)	Damaj et al., 2005
Nicotinic α4β2 antagonism	Xenopus oocytes	Voltage clamp (IC$_{50}$)	3900 (IC$_{50}$)	3000 (IC$_{50}$)	Damaj et al., 2005

NA — noradrenaline; 5-HT — serotonin.

separate study placed IC_{50} values for DM against NA uptake at 500 to 1700 nM (Rogers & Lemaire, 1991). The values from these functional studies show greater potency than the K_i for inhibition of radioligand binding at NA reuptake sites (6000 to 14,000 nM) (Table 1), and the reason for this discrepancy is not known. An investigation of DM (5 mg/kg i.p.) in non-anesthetized cats showed two-fold enhanced nictitating membrane responses to direct application of both serotonin and NA, suggesting that DM enhances the actions of both neurotransmitter systems in vivo (Sinclair, 1973). However, confirmatory studies of NA reuptake blockade from DM in humans are lacking.

3.5. Blockade of N-methyl-D-aspartate receptors

The NMDAR is one of three kinds of ionotropic glutamate receptors, which together mediate most rapid excitatory neurotransmission in the brain (Dingledine et al., 1999; Purves et al., 2001). NMDARs are cation channels that each contain four protein subunits. Overall, NMDAR channels function in a manner similar to other ligand-gated ion channels in allowing both sodium and potassium ions to flow. However, NMDARs also allow calcium to enter cells, which, in turn, activates a number of processes inside of cells (Lau & Zukin, 2007). NMDARs are unusual because their function requires two different agonists (glutamate and also the co-agonists glycine or D-serine). In addition, NMDARs have conductance that is enabled by depolarization, which reduces blockade of the channel pore by magnesium ions. Finally, NMDA receptors are required for multiple forms of synaptic plasticity, including long-term potentiation (LTP) and long-term depression (LTD) that are necessary for several kinds of memory formation (Lau & Zukin, 2007).

Electrophysiology studies in isolated cells indicate that the DM blockade of the NMDAR channel is more effective at voltages near the resting potential of the neuronal membrane than at depolarizing voltages. This voltage-dependent effect suggests that DM binds in an uncompetitive manner at a site deep within the ion channel pore, similar to other NMDAR channel blockers (e.g., ketamine, phencyclidine, or memantine) (Ferrer-Montiel et al., 1998).

The NMDAR channel blocking kinetics of DM and other uncompetitive NMDAR antagonists are summarized in Table 3 (data from Parsons et al., 1995). Rapid unbinding from the channel open state and a favorable tolerability profile differentiate DM and memantine (Bresink et al., 1995; Parsons et al., 2007) from high-affinity, slow-unblocking ligands such as phencyclidine, ketamine or MK-801, the use of which is associated with characteristic side effects of confusion, agitation, memory loss and hallucinations (Lipton, 2006; Parsons et al., 2007). This point of

differentiation is further supported by findings in rodents where DX, phencyclidine, and ketamine were found to cause large increases in spontaneous locomotor activity (a dissociative behavioral effect mediated by NMDAR block), whereas DM was found to cause a modest increase in locomotion at high doses only, which the authors stated could be a result of metabolism to DX (Szekely et al., 1991; Danysz et al., 1994). The 20% reduction of sustained NMDAR responses in cultured neocortical neurons elicited with 100 nM DM (Trube & Netzer, 1994), and the fact that the unbound concentration of DM in DM/Q-treated humans ranges from 56 nM to 96 nM, suggest that DM treatment with therapeutic doses results in only a fractional block of NMDAR channels.

In addition to the synaptically-evoked NMDAR currents, NMDAR currents from non-synaptic glutamate (at near-resting membrane potential) occur spontaneously and are thought to arise mostly from glutamate released from glial cells via nonvesicular mechanisms (Le Meur et al., 2007; Hardingham & Bading, 2010; Oh et al., 2012; Papouin & Oliet, 2014). Non-synaptic NMDAR currents may alter the functional input–output relationship of cortical pyramidal neurons (Sah et al., 1989) and also may be involved in NMDAR-dependent cell death and neurodegenerative diseases, as well as the pathophysiology of depression (Okamoto et al., 2009; Hardingham & Bading, 2010; Miller et al., 2014). Non-synaptic NMDARs are relatively enriched in the subunit GluN2B and are preferentially activated by the co-transmitter glycine rather than by D-serine (Papouin & Oliet, 2014). Furthermore, activation of nonsynaptic NMDARs appears to be necessary for long-term depression (LTD), but not for long-term potentiation (LTP) (Papouin & Oliet, 2014). Studies of the kinetically slow NMDA blocker MK-801 (Le Meur et al., 2007) and the kinetically fast compound memantine (Leveille et al., 2008; Xia et al., 2010) indicate that the competitive NMDAR antagonist d-(−)-2-amino-5-phosphonopentanoic acid (AP-5) has equal potency to block spontaneous non-synaptic and synaptic NMDAR currents. However, MK-801 (applied under conditions that activate synapses) preferentially blocks synaptic NMDARs, but memantine (and presumably also DM), due to rapid off-binding kinetics, more selectively blocks non-synaptic NMDARs (Parsons et al., 2007; Leveille et al., 2008; Hardingham & Bading, 2010; Xia et al., 2010). The impact of DM/Q treatment on NMDARs needs to be elucidated in future research to assess, for example, whether DM/Q produces fractional blockade of NMDARs at doses used therapeutically, and particularly at hyperpolarized membrane voltages and at non-synaptic NMDARs.

Furthermore, like memantine, DM/Q treatment may be slightly more effective at blocking NMDARs comprised of certain subunit combinations (GluN1/GluN2B or GluN1/GluN2C) than others (GluN1/GluN2A) (Dravid et al., 2007). In addition, the subunit selectivity of some NMDAR antagonists increases when physiological concentrations of magnesium ions (1 mM) are present (Kotermanski & Johnson, 2009). This same effect might somewhat increase the selectivity of other channel blockers including DM and DX for GluN2C- and GluN2D-containing NMDARs in comparison to GluN2B- and GluN2A-containing NMDARs. GluN1/GluN2A receptors are the most common NMDAR subtype expressed in the neocortex (Table 4).

Action at NMDARs has been used to explain various therapeutic effects of DM in humans (Verhagen Metman et al., 1998; Ilkjaer et al., 2000; Duedahl et al., 2005; Wankerl et al., 2010; Ehret et al., 2013); however, in each of these cases, other receptors could also be important. Furthermore, in humans the NMDAR block with DM administered alone may result from the metabolite DX rather than from unchanged DM. Because DM has faster dissociation kinetics than DX at NMDAR, the presumed NMDAR-mediated actions of DM given alone should be reappraised with DM/Q, which provides substantially higher DM plasma concentrations and correspondingly lower DX concentrations (see comparative NMDAR kinetics of DM and DX in Table 3). The clinical relevance of NMDAR interactions even with the altered ratio of DM to DX remains unclear.

Table 3
Potency and kinetics of channel blocking drugs on neuronal NMDA receptors.

Drug	MK-801 binding K_i (nM)	Patch clamp IC_{50} (nM)	K_{on} at IC_{50} (ms)	K_{off} (s^{-1})
MK-801	2.6	140	34,000	0.005
Phencyclidine	500	1000	13,750	0.050
Dextrorphan	NTa	1300	4762	0.075
Ketamine	200	1600	1024	0.075
Dextromethorphan	780	6100	2125	0.179
Memantine	700	2300	820	0.200

Note: For radioligand binding, purified rat synaptic membranes were incubated with [³H](+)-MK-801, and nonspecific binding was defined with 10 μM MK-801. For patch clamp recordings, isolated superior colliculus neurons from rat embryos were cultured with serum-supplemented minimal essential medium until recording. Membrane potential was held at −70 mV, and voltage-clamped responses to 200 μM NMDA were obtained with and without the indicated drug present. Patch clamp IC_{50} values were determined with at least three concentrations of test drug producing between 15% and 85% inhibition. The rates of drug association (K_{on}) and dissociation (K_{off}) were determined by measuring the rate of inhibition and recovery upon drug application and withdrawal.

  [a] NT — not tested. Data reprinted and adapted from Parsons et al. (1995) with permission from Elsevier.

Table 4
Pharmacological block of cation channels by dextromethorphan.

Cation channel type	Tissue source	DM potency	Reference
GluN1/GluN2A NMDA receptors	Recombinant cells	$IC_{50} = 11,000$ nM	Dravid et al., 2007
Serotonin 5HT$_3$ receptor channel current	Rat nodose ganglion neurons	$IC_{50} \sim 10,000$ nM	Ishibashi et al., 2006
Glycine inhibitory receptor current	Guinea pig nucleus tractus solitarius neurons	$IC_{50} \sim 3000$ nM	Takahama et al., 1997
GTPγS activated GIRK potassium current	Rat raphe neurons	$IC_{50} \sim 10,000$ nM	Ishibashi et al., 2000
Kv1.2 and Kv1.3 potassium channel electrophysiology	Cloned channels expressed in *Xenopus*	$IC_{50} = 13,000$ nM	Lee et al., 2011
Ca^{2+} fluorescence via voltage-gated Ca^{2+} channels	Cultured rat hippocampal neurons	$IC_{50} \sim 20,000$ nM	Church et al., 1991
Ca^{2+} influx via voltage-gated Ca^{2+} channels	Rat brain synaptosomes	$IC_{50} = 48,000$ nM	Carpenter et al., 1988
Voltage-gated Ca^{2+} current electrophysiology	Mouse cortex neurons	$IC_{50} = 50,000$ nM	Netzer et al., 1993
Na^+ current electrophysiology	Cultured mouse cortex neurons	$IC_{50} = 90,000$ nM	Trube & Netzer, 1994
K^+ induced $^{45}Ca^{2+}$ uptake (L-type channels)	PC12 cells	$IC_{50} = 100,000$ nM	Carpenter et al., 1988
AMPA currents	Cultured mouse cortex neurons	$IC_{50} > 10,000$ nM	Trube & Netzer, 1994

Note — None of the actions described in this table occur with free concentrations of DM found in plasma with DM/Q therapy (Fig. 4, "Cation channel block"). Therefore, none of these actions are thought to be relevant for therapy with DM/Q. Readers are referred to the cited papers for details of methods used.

3.6. Antagonist action at nicotinic α₃β₄ receptor ion channels

Two reports indicate that DM is a functional antagonist of nicotinic acetylcholine (ACh) receptors (Hernandez et al., 2000; Damaj et al., 2005), particularly those comprised of α₃β₄ subunits (IC₅₀ value of 700 nM for α₃β₄ recombinant receptors vs. 3900 nM for α₄β₂ receptors expressed in *Xenopus* oocytes) (Damaj et al., 2005). Although DM is a functional antagonist it does not compete with the agonist binding site (Hernandez et al., 2000; Werling et al., 2007b). Nicotinic ACh receptors are pentameric ion channels with several different subunits that are permeable to sodium and potassium ions. In addition, there are different subtypes of each subunit. Channels consisting of both α₃ and β₄ subunits also are permeable to calcium ions. This subtype of nicotinic ACh receptor is present on the presynaptic terminals of noradrenergic neurons in the neocortex, as shown by the block of NA release from rat neocortical synaptosomes by the selective nicotinic cone snail toxin α-AuIB (Kulak et al., 2001). There also is evidence that the α₃β₄ nicotinic subtype is the predominant mediator of ACh-induced adrenaline release from adrenal chromaffin cells (Campos-Caro et al., 1997; Tachikawa et al., 2001).

The selectivity and potency of DM at nicotinic ACh receptors is similar to that of mecamylamine, a nicotinic ACh receptor antagonist that is somewhat selective for α₃β₄ receptors. Mecamylamine reduces release of adrenaline and NA from isolated rat adrenal glands, suggesting potential moderation of some behavioral effects of stress in vivo (Yokotani et al., 2002). It is interesting that mecamylamine, in addition to its use as an antihypertensive drug, also has been used at lower dosages as therapy for Tourette's syndrome (Sanberg et al., 1998). Although not effective to reduce Tourette's symptoms, one placebo-controlled trial showed that mecamylamine was associated with a decrease in sudden mood changes in young patients (Silver et al., 2001). Mecamylamine also reduced cue-induced craving in cocaine addicts (Reid et al., 1999) and attenuated the somatic stimulant effects of alcohol (Blomqvist et al., 2002; Chi & de Wit, 2003) and tobacco use in humans (Rose et al., 1994). However, in a clinical trial, the mecamylamine enantiomer, dexmecamylamine, was not different from placebo in a multicenter add-on study of treatment for major depressive disorder (Vieta et al., 2014). Whether DM/Q treatment would have effects similar to mecamylamine is currently not known.

The role of α3β4 nicotinic ACh receptors in reducing drug-seeking behaviors has been discussed in several animal pharmacology papers (Glick et al., 2002; Taraschenko et al., 2005) and in papers cited therein. Other studies independently suggest that the nicotinic α3β4 subtype is important for modulating ACh release in brain areas known to be important for drug addiction (Grady et al., 2009).

Animal studies provide evidence that DM acts at nicotinic receptors in vivo. DM potently reduced analgesia from exogenous nicotine in the mouse tail-flick test (Damaj et al., 2005). DM was effective at lower dosages with s.c. dosing (0.8 mg/kg) than with i.p. dosing (2.4 mg/kg), suggesting that DM (less subject to first-pass metabolism with s.c. dosing),

and not DX, was the active moiety. The effect of DM was not considered to be due to an NMDAR antagonist action since MK-801 had no effect in this experiment. Although this result does not speak to possible analgesic actions of DM in mice in the absence of exogenous nicotine, it does suggest that DM reduces nicotinic responses in this model at low dosages.

In a placebo-controlled study in humans undergoing knee surgery, preoperative administration of DM (30 mg p.o.) significantly diminished tourniquet-induced hypertension and tachycardia, effects associated with sympathetic activation (Yamashita et al., 2004). Whether DM or DM/Q act to reduce sympathetic tone through α₃β₄ nicotinic receptor antagonism in humans and whether α₃β₄ antagonism has relevance for the therapeutic effect of DM/Q in the treatment of PBA requires further study.

3.7. Action of dextromethorphan at other known targets

DM has been reported in various in vitro studies to block several different cation channels including voltage-gated sodium channels, potassium channels, calcium channels and some ligand-gated ion channels such as serotonin 5-HT₃ receptors (Table 4). However, most of these IC₅₀ values are in the range of 10,000 nM and greater. For this reason, it is unlikely that even fractional channel block is obtained with the free DM concentrations that are achieved in the brain with clinical DM/Q treatment. Since the extrapolated values for unbound plasma concentrations of DM during DM/Q therapy are 35 to 120 nM, it is very unlikely that receptor actions with Ki or IC₅₀ values of ≥3000 nM in vitro would have significant clinical impact in humans. Therefore, it is also unlikely that recommended therapeutic doses of DM/Q would have any pharmacological activity at the targets in Table 4 despite the actions of DM at each of these sites in vitro.

DM inhibits glycine-gated chloride channels, which are blocked by DM with IC₅₀ and Kd values that are both near 3000 nM (Takahama et al., 1997). The action of DM at inhibitory glycine receptors is unlikely to be clinically relevant because of the relatively high concentrations needed.

DM is a lipophilic molecule with an ionizable amine at one end. This kind of molecule is partially permeable to several different cation channels, allowing open-channel block of various ion channels without much selectivity (Catterall, 1994; Gao et al., 2005). One subtype of NMDAR (GluN1/GluN2A, the most common in forebrain) is also shown in Table 4 as less likely to be blocked by DM.

4. Original analysis: estimation of dextromethorphan effects in brain based on in vitro function and unbound plasma dextromethorphan concentrations

There are no in vivo human positron emission tomography (PET) or single-photon emission computed tomography (SPECT) data to directly assess occupancy of various relevant receptors in the brain following

DM/Q treatment. Other more indirect methods must be used to estimate receptor occupancy at individual molecular drug targets. The following analysis uses calculated unbound plasma, extrapolated "CSF", or "unbound brain" drug concentrations of DM and DX relevant in the treatment of PBA for comparison with in vitro functional pharmacological results (Table 2) to arrive at an estimate of which targets may be important for DM effects in the clinical treatment of PBA. Recent analyses of the pharmacology of drugs acting within the brain at more than one molecular site have compared clinically determined unbound plasma drug concentrations to in vitro data on the functional K_i or IC_{50} values of the same drug (Derijks et al., 2008; Brynne et al., 2013). A similar approach is used in the present analysis to distinguish receptor/transporter interactions that are clinically relevant from those that may occur only at concentrations greater than those encountered clinically in treating PBA.

DM plasma concentrations have been measured in several clinical trials of DM/Q, and can be estimated in rat or mouse models by comparing doses with pharmacokinetic studies in these species (unpublished Avanir mouse pharmacokinetic study; Wu et al., 1995). Drug binding to plasma proteins also has been measured both in rats and in humans, and the fraction unbound (45% in rats and 35% in humans) does not vary with DM concentration (Witherow & Houston, 1999; Avanir Pharmaceuticals, Inc., 2010). Unbound DM plasma concentrations are consistent with values found experimentally in human CSF (Fig. 3). Therefore, extrapolated plasma concentrations of unbound DM during DM/Q therapy can be compared with DM concentrations in various in vitro functional studies (Fig. 4). At DM/Q concentrations measured during clinical treatment for PBA, DM is estimated to be present at brain interstitial fluid concentrations sufficient to inhibit serotonin and NA reuptake, partially block NMDARs, and partially block $\alpha_3\beta_4$ nicotinic receptor channels. These conclusions are based on direct interactions of drugs at extracellular receptors and do not consider the potential for

drugs to bind to intracellular receptors during early exocytotic pathways of cellular protein processing (Lester et al., 2012). Although DM reaches sufficient concentrations to bind to the intracellular Sig1-R protein, it is less clear, based on current information, whether sufficient concentrations are reached for it to act as a functional Sig1-R agonist. It is unlikely that DM would bind significantly to serotonin 5-HT$_{1B}$ receptors (Table 1) or block other cation channels (see discussion in Section 3.7) except in cases of overdose.

5. Discussion and conclusions

Although DM may be best known as an NMDAR channel blocker, several additional receptor targets may contribute to its pharmacodynamic and therapeutic effects. DM receptor interactions based on in vitro experimental results and DM plasma concentrations achieved in clinical studies of DM/Q allow us to identify which of these may be of greatest clinical relevance (Fig. 5). Our analysis suggests that DM acts as a serotonin reuptake inhibitor and as a norepinephrine reuptake inhibitor at standard clinical doses of DM/Q and would also be expected to provide fractional block of NMDAR channels with kinetics allowing more rapid dissociation and better tolerability than drugs like phencyclidine. Partial NMDAR blockade could account for decreases in late neocortical excitability seen with DM/Q treatment, as measured by auditory evoked potentials in persons with PBA (Haiman et al., 2009). Additionally, DM may provide partial block of nicotinic ACh receptor channels, particularly of the $\alpha_3\beta_4$ subtype. This subtype-selective nicotinic blocking action may contribute to reduced cholinergic activation following emotionally relevant stimuli. Finally, DM may act as a Sig1-R agonist resulting in the modulation of one or more neuronal ion channels, monoamine and glutamate-mediated transmission, or other intracellular actions (Maurice & Su, 2009) which could be relevant for PBA treatment. Data with DM for other potential targets (e.g., block of
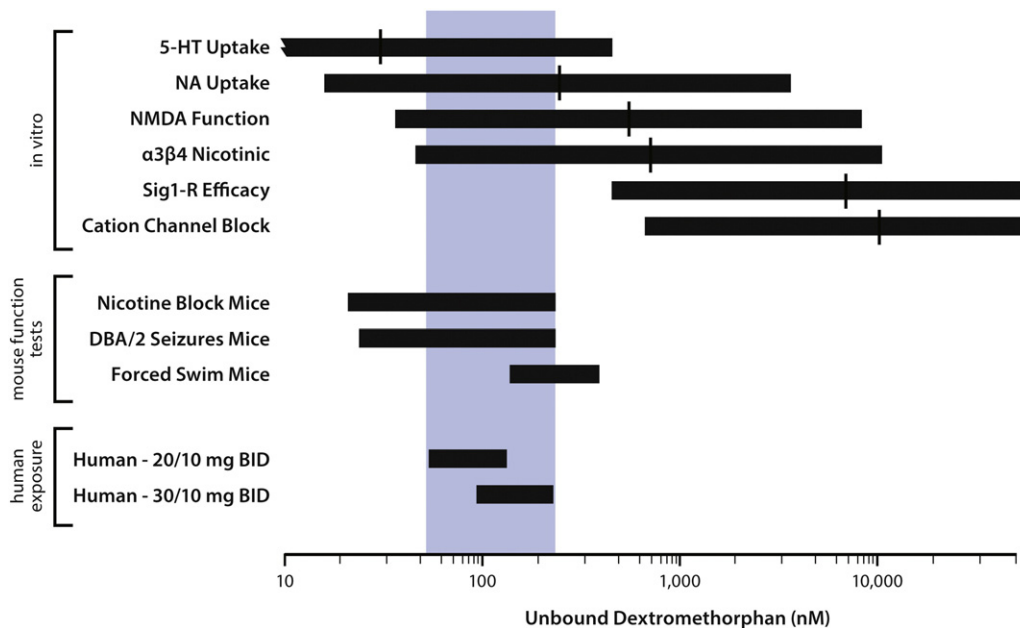
Fig. 4. Effective concentrations of DM (from in vitro pharmacology studies, top) are compared to free plasma drug concentrations (equal to CSF concentrations) from mouse studies (middle) and clinical trials of DM/Q (bottom). In vitro pharmacological IC_{50} values (black horizontal bars) are from the following references: serotonin (5-HT) uptake and noradrenaline (NA) uptake (Codd et al., 1995); NMDAR block in cultured neurons (Trube & Netzer, 1994); nicotinic receptor subtype $\alpha_3\beta_4$ block (Damaj et al., 2005); Sig1-R efficacy against rat spreading depression (Anderson & Andrew, 2002); cation channel block (see Table 4). The range of concentrations shown for in vitro function (thick horizontal bars) represent IC_{50} or K_i (vertical bar) with 15-fold ranges greater or less, illustrating the dynamic range between ~10% and 90% effect from the Hill equation. In vivo mouse data are from the following references: nicotine block (Damaj et al., 2005); DBA/2 seizures (Chapman & Meldrum, 1989); antidepressant-like action (forced swim test) (Nguyen et al., 2014). Free drug concentrations in mice were determined from ED$_{50}$ doses, pharmacokinetic peak plasma concentrations, and 55% plasma binding in rats (Witherow & Houston, 1999). Human exposure (free concentrations) was determined by accounting for 65% of drug bound nonspecifically to plasma proteins in clinical samples (Avanir Pharmaceuticals, Inc., 2010). The pale blue box shows the range of free DM in human plasma (C$_{max}$) from clinical trials when given at the dose specified (DM dose/Q dose given twice daily, BID). Clinical data represent free drug concentrations following clinical trials of DM/Q. For 20 mg/10 mg and 30 mg/10 mg BID doses, data are the 95% confidence interval of mean C$_{max}$. Human data are from unpublished Avanir pharmacokinetic trial 13-AVR-134.
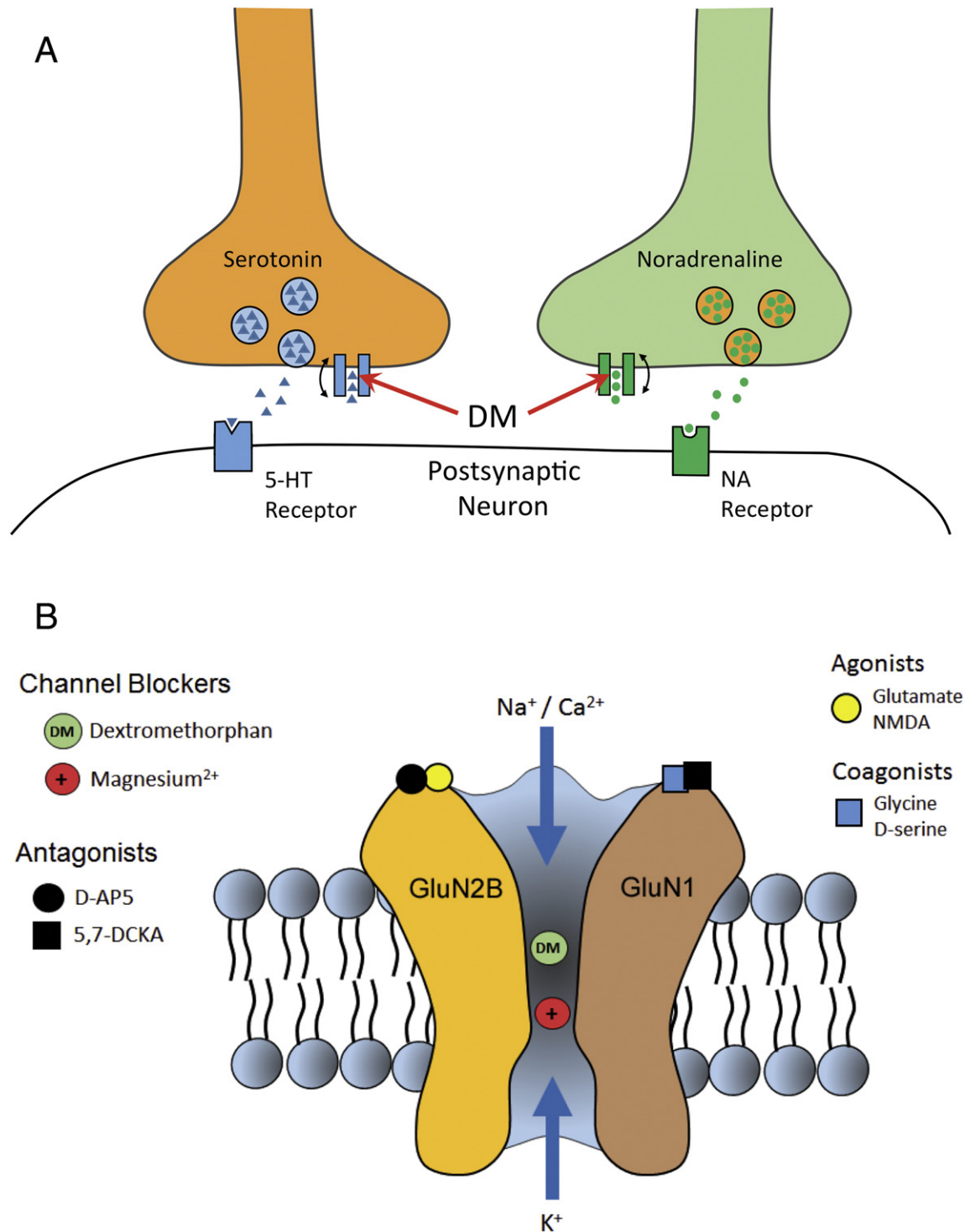
Fig. 5. Summary of DM molecular sites of drug action in human brain. (**A**) DM inhibits the action of serotonin transporters and NA transporters similar to a well-known class of drugs. (**B**) DM binds as a readily-reversible blocker of NMDA-type glutamate receptors at a site (DM) similar to those of memantine, ketamine and phencyclidine and not far from the site of endogenous Mg^{2+} ion binding. At this site, DM prevents permeant ions (sodium, calcium and potassium) from producing ionic currents and also limits increases in intracellular calcium concentration. However, the dissociation rate of DM from NMDA receptors is quite rapid, which may be important for tolerability in patients (see text for details). Figure adapted from Parsons et al. (2007) with permission from Elsevier. (**C**) DM blocks nicotinic acetylcholine-gated ion channels by binding within the channel pore. Although DM blocks several subtypes of nicotinic receptors, its action is somewhat selective for receptors composed of $\alpha3$ and $\beta4$ subunits. This binding prevents the influx of Na^+ and Ca^{2+} and reduces excitation of neurons from acetylcholine. Figure adapted from Changeux (2010) with permission from Elsevier. (**D**) DM acts as a Sig1-R agonist in vitro, and animal data also support this finding (antidepressant-like and anticonvulsant activity are blocked by sigma antagonists). The molecular changes that result from Sig1-R actions of DM are not clear. This figure shows the Sig-1R protein chaperone as a cytosolic and plasma membrane signaling molecule. At the interface between endoplasmic reticulum (ER) and mitochondrion (MAM or mitochondrion-associated membrane), activation by Sig-1R agonists causes Sig-1R to dissociate from an ER chaperone (BiP). Subsequently, Sig-1R associates with the inositol trisphosphate receptor (IP3R) to enhance Ca^{2+} release from ER into mitochondria, modulating mitochondrial function. If stimulated by high concentrations of Sig-1R agonists ($10\times$ the radioligand binding K_i value) or in the presence of strong ER stress, Sig1-Rs rapidly migrate from MAM and become associated with ion channels at the plasma membrane where they modulate channel expression and function. In different cell types, these Sig-1R associated proteins include NMDA receptors, ion channels (NaV1.5, ASIC, hERG, Kv1.4, Kv1.5, SK) and potentially other proteins. Figure adapted from Su et al. (2011).
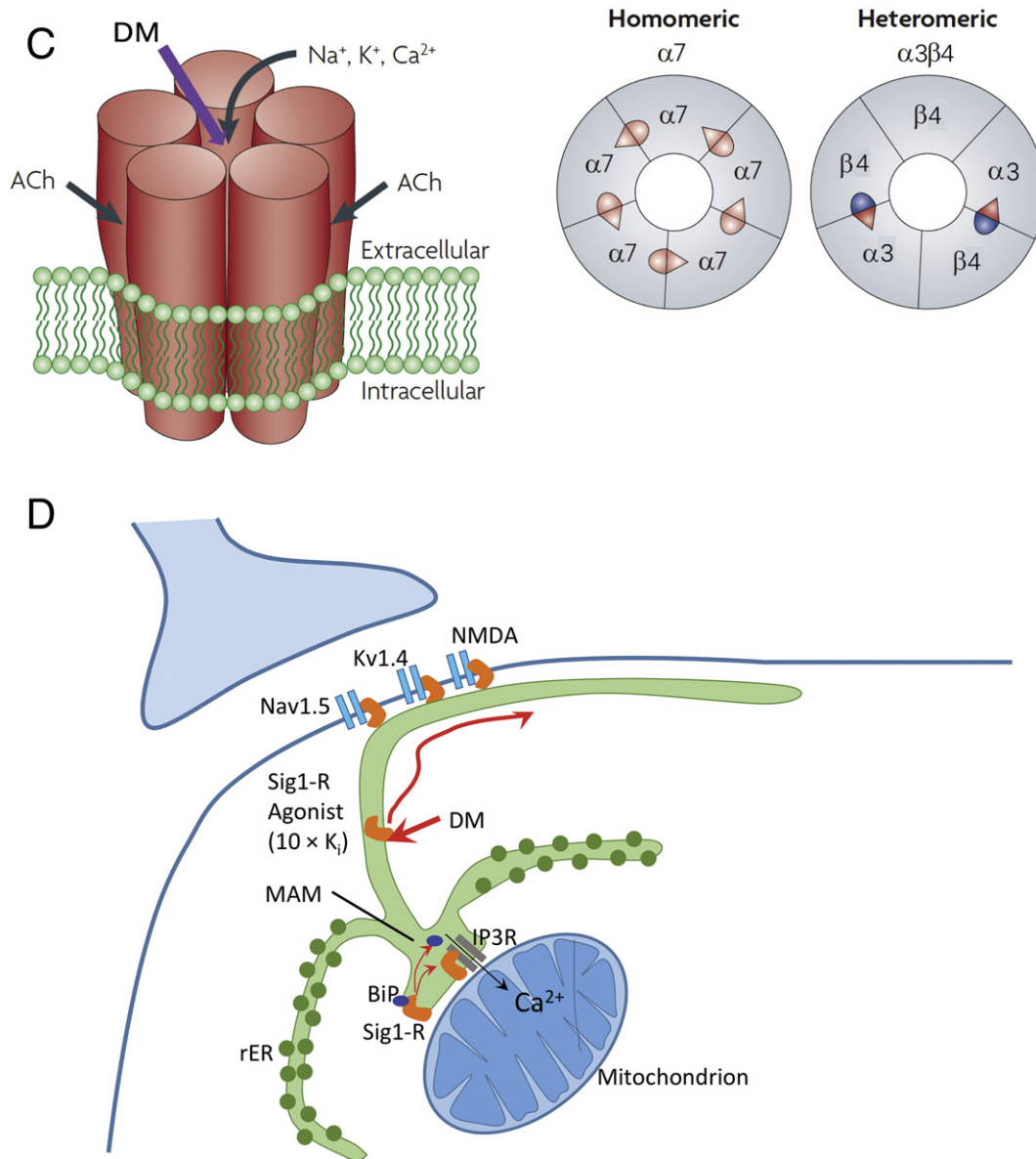
Fig. 5 (*continued*).

voltage-gated sodium and voltage-gated calcium channels) suggest minimal or insignificant interactions of DM/Q at currently used doses.

Additional study will be required to confirm the extent and time course of DM receptor occupation at various sites in the human brain, and the extent to which activity at each of these sites may contribute to the clinical efficacy of DM/Q for PBA. Future experimental studies with specific positron emitter tracer ligands or similar methods in human subjects treated with DM/Q would be particularly useful to test the extent that DM binds to these receptor targets in human brain. Such approaches have been used previously to study human drug receptor occupancy to various receptor and transporter targets in vivo (Mamo et al., 2007; Pike, 2009).

The receptor sites of DM/Q treatment in the brain that we have identified are consistent with the proposed pathophysiology of PBA. The details of pathophysiology in PBA are beyond the scope of this review. However, it is interesting that the receptors that we have identified as likely targets of DM/Q treatment are among those that have been implicated based on the functional anatomy of the brainstem and descending pathways to the brainstem that are relevant for PBA (Parvizi et al., 2001; Parvizi et al., 2009; Lauterbach et al., 2013; Cummings et al., 2015).

In summary, our analysis indicates that the addition of quinidine to dextromethorphan greatly increases the amount of circulating free dextromethorphan and decreases the metabolite dextrorphan, to the extent that the main active drug moiety with DM/Q treatment in humans is dextromethorphan. Furthermore, we have shown that the amount of free dextromethorphan achieved with DM/Q treatment is sufficient to significantly inhibit serotonin and noradrenaline transporters in human brain and also sufficient to bind at Sig1-R and to partially block ion channels gated by certain NMDAR and nicotinic acetylcholine receptors. One or more of these potential therapeutic targets are likely to be involved in the clinical actions of DM/Q treatment.

Conflict of interest statement

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