2013

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10.1038/tp.2013.97
This article was originally published as: P R Bharadwaj, K A Bates, T Porter, E Teimouri, G Perry, J W Steele, S Gandy, D Groth, R N Martins, and Giuseppe Verdile., Latrepirdine: molecular mechanisms underlying potential therapeutic roles in Alzheimer's and other neurodegenerative diseases. *Translational Psychiatry* (2013) 3, e332; doi:10.1038/tp.2013.97 Original article available here

OPEN

Citation: Transl Psychiatry (2013) 3, e332; doi:10.1038/tp.2013.97

www.nature.com/tp

REVIEW

Latrepirdine: molecular mechanisms underlying potential therapeutic roles in Alzheimer's and other neurodegenerative diseases

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Latrepirdine (Dimebon™) was originally marketed as a non-selective antihistamine in Russia. It was repurposed as an effective treatment for patients suffering from Alzheimer's disease (AD) and Huntington's disease (HD) following preliminary reports showing its neuroprotective functions and ability to enhance cognition in AD and HD models. However, latrepirdine failed to show efficacy in phase III trials in AD and HD patients following encouraging phase II trials. The failure of latrepirdine in the clinical trials has highlighted the importance of understanding the precise mechanism underlying its cognitive benefits in neurodegenerative diseases before clinical evaluation. Latrepirdine has shown to affect a number of cellular functions including multireceptor activity, mitochondrial function, calcium influx and intracellular catabolic pathways; however, it is unclear how these properties contribute to its clinical benefits. Here, we review the studies investigating latrepirdine in cellular and animal models to provide a complete evaluation of its mechanisms of action in the central nervous system. In addition, we review recent studies that demonstrate neuroprotective functions for latrepirdine-related class of molecules including the β-carbolines and aminopropyl carbazoles in AD, Parkinson's disease and amyotrophic lateral sclerosis models. Assessment of their neuroprotective effects and underlying biological functions presents obvious value for developing structural analogues of latrepirdine for dementia treatment.

Translational Psychiatry (2013) **3,** e332; doi:10.1038/tp.2013.97; published online 3 December 2013

Keywords: Alzheimer's disease; latrepirdine; mechanism of action

INTRODUCTION

With an ageing population, there is a need for both preventative and disease-modifying treatments for Alzheimer's disease (AD). Current AD treatments target cognitive decline and provide only minor benefits across the array of clinical symptoms.[1,2] Approved AD drugs such as acetylcholine esterase (ACE) inhibitors (donepezil, rivastigmine and galanthamine) and *N*-methyl-D-aspartate (NMDA) receptor antagonists (Memantine) are generally prescribed as monotherapy or in combination. However, approved AD drugs are expensive and, most importantly, do not prevent disease progression and are of limited benefit to most patients.[3,4]

Latrepirdine was initially used in Russia in 1983 as a non-selective antihistamine for the treatment of skin allergy and allergic rhinitis.[5–7] Studies outlining the significance of antihistamine drugs in treating neurodegenerative disorders and the neuroprotective functions of latrepirdine in animal models initiated interest for repurposing latrepirdine as a potential therapy for AD and Huntington's disease (HD). Despite the lack of understanding of latrepirdine's mechanism of action in the central nervous system, it was evaluated in clinical trials for AD[8] and HD[9] (Table 1). A pilot clinical trial performed on 14 patients with mild–moderate AD supported the results of the cellular and animal studies. Significant improvement in both cognitive function and psychiatric symptoms was observed in all patients undergoing latrepirdine treatment. Also, a distinct antidepressive effect was observed in AD patients.[5] In 2008, data from a phase II randomized, double-blind, placebo-controlled study conducted in Russia demonstrated for the first time, improvement across a range of clinical outcomes due to a therapeutic intervention in mild–moderate AD.[8] Phase III trials, CONNECTION and CONTACT were then launched and results released in early March 2010 showed no improvement in any primary or secondary outcome measures of cognition in the patients.[10] Similar to the outcomes in AD clinical trials, phase III trial of latrepirdine in HD patients failed to show efficacy in the 6-month HORIZON trial, which enrolled 403 people with HD in 11 countries.[11] In contrast to the cognitive benefits observed in phase II trials, no significant improvements over placebo-treated patients were observed in HD patients administered latrepirdine.[9]

It is evident that latrepirdine trials in HD and AD have progressed to phase III trials based on an encouraging phase II trial data. It is although uncertain whether the failure of latrepirdine in phase III can be attributed to the poor efficacy of the drug or due to the lack of trial optimization. The benefits of

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Received 5 August 2013; revised 19 September 2013; accepted 26 September 2013

Table 1. Latrepirdine clinical trials for Alzheimer's, Huntington's disease and schizophrenia

Trial name and country	*Number of participants*	*Patient type*	*Average age/MMSE at baseline*	*Treatment regimen*	*Primary end points (functional)*	*Outcome*	*Reference*
Russia (pilot/phase I)	14	Mild–moderate AD	64–68 years	8 weeks, 20 mg t.i.d.[a]	Hazegawa scale (MMSE equivalent)	Significant improvement of cognitive and self-service functions	5
Russia (phase II)	11 sites $n = 183$	Mild–moderate AD	68 years/18.7	6 months, $N = 89$ on 20 mg t.i.d. $N = 94$ on placebo	ADAS-cog CIBIC-plus	Significant improvement in cognition over placebo and baseline levels	8
CONNECTION (phase III) North and South America, Europe	63 sites $n = 598$	Mild–moderate AD	74.4 years/17.7	6 months $N = 1/3$ 5 mg t.i.d. $N = 1/3$ 20 mg t.i.d. $N = 1/3$ placebo	ADAS-cog CIBIC-plus	No change in any primary or secondary outcome measures	83
CONCERT USA, UK, Europe, Australia and New Zealand	$n = 1050$	Mild–moderate AD on donepezil	50 years and older/12–24	12 months $N = 1/3$ 5 mg t.i.d. $N = 1/3$ 20 mg t.i.d. $N = 1/3$ placebo	ADCS-ADL ADAS-cog	No change in any primary or secondary outcome measures	83,84
CONTACT Europe and South America	N/A	Moderate-to-severe AD on donezepil	Terminated	6 months 20 mg t.i.d. Placebo	N/A	N/A	10
CONSTELLATION	N/A	Moderate-to-severe AD on memantine	Terminated	N/A	N/A	N/A	85
DIMOND US and UK (phase II)	17 sites $n = 91$	Mild-to-moderate HD	52–53 years/25.1–25.6	90 days $N = 46$ 20 mg t.i.d. $N = 45$ placebo	UHDRS MMSE ADAS-cog	No change in UHDRS, significant improvement in cognition (MMSE only)	9
HORIZON North America, Europe and Australia	64 sites $n = 403$	Mild-to-moderate HD (>30 years of age, $\geqslant 36$ CAG polyglutamate repeat expansion)	30 years and older	6 months 20 mg t.i.d. Placebo	MMSE CIBIC-plus	No change in any primary or secondary outcome measures	11
Russia (phase II)	$n = 56$	Paranoid schizophrenia on risperidone	35–50 years	4–8 weeks 4–6 mg risperidone $N = 29$ 20 mg t.i.d. $N = 27$ Placebo	PANSS, CGI-S	Improvement of negative symptoms and certain components of cognitive dysfunction	86

Abbreviations: AD, Alzheimer's disease; ADAS-cog, Alzheimer's Disease Assessment Scale-cognitive subscale; ADCS-ADL, Alzheimer's disease co-operative study-activities of daily living; CGI-S, Clinical Global Impression-Severity Scale; CIBIC: Clinician's Interview Based Impression of Change-Plus Caregiver Input; HD, Huntington's disease; MMSE, mini mental state examination; N/A, not available; PANSS, Positive and Negative Syndrome Scale; t.i.d., three times daily; UHDRS, Unified Huntington's Disease Rating Scale. [a]Note, no placebo control arm was used in this study.

latrepirdine in the phase II AD study were not driven by worsening in the placebo group, but reflected an absolute improvement on latrepirdine as well as a decline on placebo. In the phase III trial, however, the patients in the trial did not deteriorate significantly in either the drug-treated group or the placebo group, which makes interpretation of the study more difficult. There are also some notable differences between the Russian phase II study and the multinational phase III studies including the formulation of latrepirdine, baseline mini mental state examination (MMSE) and the age of the patients recruited (mean age 68.1 years in phase II versus 74.4 years in phase III).[8,10] Unfortunately, concerning the disease-modifying activity of latrepirdine, no brain amyloid load (Pittsburgh Compound-B-positron emission tomography (PIB-PET) imaging) or biomarker data are available from the different clinical trials as only ADAS-cog (Alzheimer's Disease Assessment Scale-cognitive subscale) was used as primary outcome.

One of the other major criticisms for the mixed outcomes from the AD and HD clinical trials was directed toward the lack of optimization of the trial design and the lack of clarity in the drug's mechanism of action responsible for its cognitive-enhancing properties. Several possible neuroprotective functions for latrepirdine have been postulated; however, there is a need to rigorously test and verify the mechanisms of action of latrepirdine and establish the dose that is most likely to be effective.[12,13] In addition to its well-known antihistamine properties, studies have demonstrated cognitive-enhancing properties in non-transgenic healthy animal models and in neurodegenerative models.[5,14–16] Studies have reported latrepirdine's ability to inhibit a number of neurotransmitter receptors, modulate Ca^{2+} metabolism, protect mitochondrial function[5,17,18] and more recently its ability to modulate intracellular catabolic pathways and reduce amyloid aggregates and pathology in cellular and mice models.[15,19–22] Unfortunately, target validation and the magnitude of the impact of these putative actions on key disease processes and clinical symptoms have not been robustly clarified. Here, we review the studies investigating the different mechanisms of action of latrepirdine in cellular and animal models, to evaluate its potential role in the treatment of AD and other neurodegenerative diseases.

MECHANISMS OF ACTION

Antihistamine and cognitive properties

Latrepirdine blocks H1 histamine receptor activity and hence was used as an antihistamine drug for the treatment of burns, allergic rhinitis and skin allergy in Russia since 1983. However, its use as an antihistamine declined with the discovery of more selective drugs.[6,23] Studies also have reported antiarrhythmic properties and an effect on coronary blood flow and myocardial contractibility,[24,25] even though the possible underlying mechanisms have not yet been determined. More recently, interest in latrepirdine rebounded following a growing interest in the role of histamine receptors (5-hydroxytryptamine 6 (5-HT6) and 5-HT7) in learning and cognitive functions[26,27] and studies reporting cognitive-enhancing and neuroprotective functions of latrepirdine.[5,15] In addition to blocking the histamine receptor activity, latrepirdine has been shown to interact with calcium channels and a wide range of other neurotransmitter receptors.

Multireceptor activity and modulation of calcium channels

Neurotoxicity and loss of neuronal processes caused by excitatory amino acids, glutamate and aspartate is a characteristic feature of several neurodegenerative diseases. The toxic action of glutamate and aspartate is largely receptor-mediated leading to an increase in the intracellular Ca^{2+} concentration that triggers a cascade of pathological reactions causing the death of nerve cells.[28,29] Studies indicate that intracellular calcium (Ca^{2+}) flux is critical in synaptic plasticity, a cellular mechanism for learning and memory. Therefore, modulators of Ca^{2+} channels and receptor functions regulating calcium influx are currently a focus of interest for their potential use as neuroprotective agents.[30–32] In the Bachurin *et al.*[5] study, latrepirdine suppressed Ca^{2+}-induced contractions of smooth muscles in a reversible and concentration-dependent manner. Similar effects of latrepirdine were observed in neuronal cultures from wild-type mice and HD transgenic mice, which harbour the entire human *HD* gene containing 128 CAG repeats. Also, latrepirdine was found to act as an inhibitor of NMDA receptors and voltage-gated calcium channels. Application of latrepirdine stabilized glutamate-induced Ca^{2+} signals and conferred protection from glutamate-induced apoptosis.[16]

In one of the initial studies, the effects of latrepirdine on AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and NMDA subtypes, glutamate receptors in rat cerebral neurons, were evaluated in a comparative study against memantine, a potent NMDA receptor antagonist.[33] Both latrepirdine and memantine in low concentrations potentiated activity of AMPA receptors in rat cerebellar Purkinje cells. In rat cortical neurons, both latrepirdine and memantine efficiently blocked NMDA receptor activity with different potencies. The differences in the effects of memantine and latrepirdine may be determined by their interaction with different channel and/or receptor subunits of NMDA receptors. For example, the polyamine site of the NMDA receptor NR2B subunit[34] has been suggested as a possible binding site for latrepirdine.

Interactions between latrepirdine and a number of molecular targets including ACEs, α-adrenergic, serotonergic and dopaminergic receptors have been reported.[5,33,35,36] These receptors are widely distributed in the brain and are associated with different neuropsychiatric symptoms[37,38] including hallucinations and depression in AD patients.[39,40] Evaluation of latrepirdine against a set of biochemical targets indicated that it inhibits α-adrenergic receptors (α1A, α1B, α1D and α2A), histamine H1 and H2 receptors and serotonin 5-HT2c, 5-HT5A and 5-HT6 receptors.[16,41]

In a recent study, molecular pharmacology profiling of latrepirdine was performed on a panel of 70 targets including enzymes, ion channels, neurotransmitter transporters and G-protein-coupled receptors to characterize the spectrum of its activity.[17] In addition to histaminergic receptors, latrepirdine exhibited high affinity to a range of other receptors; specifically, serotonergic, α-adrenergic and dopaminergic receptors. Latrepirdine was found to interact with relatively low affinity with some ion channels (benzothiazepine site of L-type Ca^{2+} channel, site 2 of sodium channel and hERG (human ether-a-go-go-related gene) potassium channel) and the norepinephrine transporter. Owing to its broad spectrum activity on many therapeutically important neuronal receptors, it is unclear how latrepirdine's molecular pharmacology relates to its multifunctional effects on different aspects of central nervous system activity.

Neuroprotective and cognitive-enhancing functions in animal models

Latrepirdine has been shown to possess neuroprotective functions and improve memory in animals with drug-induced cognitive impairment. In the initial work by Shadurskaia *et al.*,[42] latrepirdine was shown to possess neuroactive functions including its ability to inhibit monoamine oxidase deaminating dopamine and serotonin, thereby increasing dopamine and noradrenaline in the rat brain.[42] It was shown that latrepirdine is a potent competitive and reversible inhibitor of both ACE and butyrylcholine esterase, which are key enzymes associated with the degradation of acetylcholine in the brain.[43]

Systemic administration of latrepirdine restored memory as determined by two-way active avoidance performance in rats

injected with AF64A (L-ethyl-1-(2-hydroxyethyl) aziridinium, a neurotoxic analogue of acetylcholine that causes neurodegenerative changes characteristic of AD.[44] The data suggested that latrepirdine activated compensatory mechanisms against chronic partial deprivation of cerebral cholinergic functions, which may be responsible for the prevention of neurodegeneration in AF64A-injected rats.[15] In a subsequent study, latrepirdine showed a dose-dependent prevention of convulsions and death caused by NMDA-induced toxicity in mice and protected cerebellar granule cells against β-amyloid 25–35 (Aβ25–35), a neurotoxic fragment of Aβ protein.[5] Latrepirdine also increased survival rate of serum-starved differentiated neuroblastoma cells.[18]

In a more recent study, latrepirdine administered before methamphetamine significantly reduced the amount of striatal dopamine depletion in mice, suggesting that latrepirdine may be exerting a neurotoxin-specific protective effect.[45] Besides its neuroprotective effects, administration of latrepirdine has also shown to promote hippocampal-dependent learning in both appetitive and inhibitory tasks in mice.[46] However, in a more recent report, latrepirdine failed to exert any effect on the age-related impairment in spatial learning and performance assessed using the Morris water maze. Latrepirdine also showed no effect on the age-related increase in hippocampal expression of markers of microglial and astroglial activation in rats.[47] This study suggests that the cognitive-enhancing and neuroprotective properties of latrepirdine may be limited to the degeneration of specific neuronal functions and associated cognitive abilities. In addition to rodent models, latrepirdine has been shown to enhance memory and learning in a primate model. Acute latrepirdine administration was associated with modest improvements in the performance of a delayed matching to sample task in young adult and aged monkeys and showed a trend towards attenuation of scopolamine-induced impairments in young adult monkeys.[48]

Recent studies have provided further insight into the potential mechanisms of action that may underlie latrepirdine's cognitive-enhancing properties. Acute oral administration of latrepirdine-enhanced cognition in rats but did not alter the activity of acetylcholine (ACE) nor block NMDA-induced calcium influx, suggesting that the cognition-enhancing effects of latrepirdine are unlikely to be mediated by ACE inhibition or NMDA receptor antagonism.[49] Latrepirdine was compared against SB-399885 (selective 5-HT6 antagonist[50]) to evaluate the role of anti-histamine activity to cognitive functions. The affinity and potency of latrepirdine for 5-HT6 receptor were significantly lower than that observed with the selective 5-HT6 antagonist SB-399885. Consistent with its activity at the 5-HT6 receptor, latrepirdine was significantly less potent than SB-399885 in the improvement of memory functions.[41] It is important to note that latrepirdine interacts with several other receptor molecules and that these interactions have not been fully characterized. As many of these targets significantly impact cognitive function, the relative role of 5-HT6 receptor antagonism in the clinical efficacy of latrepirdine remains speculative. Latrepirdine was also found to be proneurogenic in an *in vivo* screen in adult mice.[19] Although it showed lower activity compared with the lead compound P7C3, an aminopropyl carbazole, latrepirdine administration showed significant increase in hippocampal neurogenesis in the mouse model.[19] This study provided further evidence for latrepirdine's cognitive-enhancing properties; however, no specific target or mechanism responsible for its actions was indicated.

Latrepirdine treatment in neurodegenerative disease models

Recent studies provide evidence for a neuroprotective effect of latrepirdine in transgenic mouse models of neurodegenerative disease. Latrepirdine has been shown to modulate amyloid pathology, reduce memory deficits in transgenic AD mice and protect against Aβ toxicity in cultured cells.[5,18,51] TgCRND8 mice[52] treated with latrepirdine exhibited a trend towards cognitive and behavioural improvement without affecting the levels of total Aβ in the brain.[53] In our recent work, TgCRND8 mice were administered with latrepirdine and subjected to behaviour analysis in the cued and contextual fear conditioning paradigm, as well as immunohistological and biochemical analysis of AD-related neuropathology. Latrepirdine treatment was associated with improved learning behaviour and with a reduction in the accumulation of Aβ42 and α-synuclein.[21,22]

The effects of latrepirdine have also been studied in other neurodegenerative disease models including Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS). In an overexpressing synuclein mice model (Thy1mγSN), chronic administration of latrepirdine was shown to significantly reduce the development of motor dysfunction and coordination.[14] However, a more recent study showed no improvement in motor skills or changes in the levels of striatal dopamine or α-synuclein in the brains of transgenic mouse model characterizing early-stage PD.[54] Also, latrepirdine did not block 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-mediated cell death of dopaminergic neurons in *Caenorhabditis elegans* or in the substantia nigra of adult mice.[55] Chronic latrepirdine administration was studied in a mouse model of γ-synucleinopathy characteristic of the pathological features of ALS. Significant improvement of motor performance, reduced amyloid inclusions, decreased amount of insoluble γ-synuclein species and a notable amelioration of astrogliosis were observed in the latrepirdine-treated animal groups.[56] However, in a more recent study, latrepirdine did not protect the spinal cord motor neurons from cell death or preserved the motor function in a mouse model of ALS.[57] Latrepirdine treatment also conferred no protection against the toxicity associated with expression of ALS-associated genes *TDP43* and *FUS*, and the HD-associated protein huntingtin with a 103 copy-polyglutamine expansion (htt-103Q) in yeast. These results indicate that latrepirdine's neuroprotective functions are limited to specific neurodegenerative mechanisms. Further analysis of latrepirdine's proposed neuroprotective effects and pathological markers including the accumulation of the misfolding protein, levels of neurotransmitters and receptor activities in these disease models can provide a greater insight into its mechanisms of action *in vivo*.

There is increasing evidence describing latrepirdine's neuroprotective effects, but the mechanisms underlying these cognitive benefits have remained elusive. During the past few decades, the concept of multitarget drug activity has been proposed.[58,59]. On the basis of this concept, the mechanisms responsible for the beneficial actions of latrepirdine may be attributed to multiple downstream effects on a number of receptors leading to change in neurotransmitter levels.

Mitochondrial activity

Mitochondrial dysfunction is a common feature of several neurodegenerative diseases including AD. In the AD brain, there is a characteristic decrease in the number of mitochondria in neurons accompanied by decreased brain glucose metabolism,[60] and reduced activities of both tricarboxylic acid cycle enzymes[61] and cytochrome *c* oxidase.[62,63] A number of studies have reported the protective effects of latrepirdine on mitochondrial structure, function and metabolism, some of which have also been indicated as the underlying mechanism of its proneurogenic properties. In the initial report, latrepirdine was shown to modulate the mitochondrial permeability transition pore (PTP). PTP regulates the transport of Ca^{2+} and smaller compounds in and out of the mitochondria. Latrepirdine suppressed the opening of PTP by neurotoxins including MPP+ (1-methyl-4-phenylpyridinium), Aβ25–35, phosphate ions, calcium ions or by butylhydroxy-peroxide in isolated rat liver mitochondria.[35] In the same study, latrepirdine was shown to suppress lipid peroxidation induced by

either butylhydroperoxide or by Aβ25–35. Although no direct evidence for the relationship between suppression of lipid peroxidation and inhibition of PTP opening was identified, these properties may be a contributing factor in the protection of mitochondrial function and in the overall neuroprotective effect of the latrepirdine.[35] In a more recent study, similar effects of latrepirdine on calcium-induced permeability transition were observed in the mitochondria isolated from rat brain. Latrepirdine attenuated Ca^{2+}-induced swelling but did not alter cytochrome *c* release or Ca^{2+} uptake capacity. The findings showed that latrepirdine reduced mitochondrial swelling without inducing any major changes in permeability transition.[64]

In primary mouse cortical neuronal cells and human neuroblastoma cells, latrepirdine improved mitochondrial function on aspects such as mitochondrial membrane potential and ATP synthesis, although the drug's effect on PTP opening was not determined.[18] Also, no change in the mitochondrial DNA copy number was observed with latrepirdine treatment, implying no effect on mitochondrial biogenesis.[18]. In an *in vivo* screen for search of novel proneurogenic compounds, latrepirdine was shown to protect mitochondrial membrane integrity at high concentrations.[19] In a more recent study, the effects of latrepirdine were tested on mitochondrial function and dynamics in a cellular model, overexpressing neurotoxic Aβ peptides. Here, latrepirdine was shown to protect against the toxic effects of Aβ on mitochondrial morphology, respiratory chain complex activities and enlarged mitochondrial mass.[65] Further support for the potential role of latrepirdine in the enhancement of mitochondrial function and subsequent energy homeostasis comes from one study that showed latrepirdine to be significantly enhanced by cerebral glucose utilization in aged mice, whereas no effect on CGU was observed in the young mice.[66]

Regulate protein aggregation

Progressive accumulation of misfolded or aggregate proteins in the brain is a characteristic feature of neurodegenerative diseases including ALS (TAR DNA-binding protein 43, TDP-43), PD (synuclein), HD (Huntington protein, mHTT) and AD (beta amyloid, Aβ). Agents that reduce aggregation and accumulation of amyloidogenic proteins in the brain have shown to improve or arrest the cognitive deficits and behavioural symptoms in animal models.[67–69] Recent studies have suggested that the ability to modulate accumulation or generation of protein aggregates in the brain may contribute to the neuroprotective and cognitive-enhancing functions of latrepirdine.

The effects of latrepirdine on the formation of TDP-43 aggregates were investigated in human neuroblastoma cells. Latrepirdine treatment reduced the number of TDP-43 aggregates as measured by immunoblot analysis. In combination with methylene blue, latrepirdine treatment further reduced the level of aggregates in cells.[70] Latrepirdine treatment significantly reduced the number of amyloid deposits in the spinal cord associated with reduced development of motor dysfunction and astrogliosis in the Thy1mγSN mouse model.[14] In a more recent study, the effects of latrepirdine on the formation of fibrillar detergent-insoluble structures formed by GS protein (the main component of pathological intracellular incorporations in the neurons of Thy1mγSN mice) were studied.[71] This study revealed a significant reduction of aggregated synuclein forms in specimens of spinal cord tissue from latrepirdine-treated mice. However, no change in the levels of intermediate protein aggregation products, oligomers and protofibrils was observed with latrepirdine treatment.

In a more recently published work, the effects of latrepirdine upon Aβ, tau and astrogliosis in the hippocampus of triple transgenic (3 × Tg-AD) mice was evaluated. A significant reduction in hippocampal/subicular APP/Aβ in latrepirdine-treated mice was shown; however, no change in the levels of full-length APP, soluble Aβ1–40 and Aβ1–42, Aβ oligomers, glial fibrillary acidic protein, beta-site APP cleaving enzyme 1 and hippocampal tau levels was observed. Interestingly, the number of the hippocampal APP/Aβ plaques in latrepirdine-treated mice was higher compared with control mice.[20] It is possible that latrepirdine accelerates the deposition of insoluble Aβ into plaques without changing the levels of soluble Aβ species.[53] Overall these findings provide evidence for latrepirdine's ability to modulate protein aggregation. Although there is currently no evidence of latrepirdine's ability to significantly alter amyloid structure, recent studies have identified other metabolic functions that may be responsible for its ability to modulate protein aggregation. In a study by Steele *et al.*,[72] latrepirdine treatment elevated levels of Aβ in the extracellular media in mouse N2a neuroblastoma cells and in isolated synaptoneurosomes from AD transgenic mice (TgCRND8). In addition, an acute dose of latrepirdine administered intraperitoneally led to an increased Aβ concentration in the interstitial fluid of AD transgenic mice. This study showed a surprising association of acute latrepirdine dosing with elevated levels of extracellular Aβ. It is suggested that latrepirdine-induced changes in neurotransmission, coupled with altered synaptic activity may account for the rapid changes in extracellular Aβ levels. Recent reports have shown that synaptic activity can dynamically alter interstitial fluid Aβ levels *in vivo*; however, the specific mechanisms responsible for latrepirdine's effects are unclear.[72–74] In addition to modulating APP/Aβ metabolism, recent reports have shown latrepirdine to enhance activity of intracellular protein degradation pathways such as autophagy.

Latrepirdine modulates protein degradation pathways

The autophagy–lysosome system represents a main intracellular degradation pathway for clearance of protein aggregates and damaged organelles in eukaryotic cells.[75] Modulating the cellular degradation pathways to enhance clearance of protein aggregates is gaining interest as a therapeutic strategy in several neurodegenerative disorders featuring abnormal protein accumulation. In our recent report, a yeast model (*Saccharomyces cerevisiae*) was used to investigate whether latrepirdine can modulate autophagy and reduce levels of Aβ42 aggregates. It was shown that latrepirdine upregulated yeast autophagic markers including vacuolar (lysosomal) activity and increased transport of Atg8 (autophagy-related protein 8) to the vacuole. Using an *in vitro* GFP-tagged Aβ yeast expression system, we showed that latrepirdine significantly reduced GFP-Aβ42 in wild-type compared with the autophagy-deficient mutant (Atg8Δ). Further, latrepirdine treatment attenuated Aβ42-induced toxicity in wild-type cells but not in the Atg8Δ mutant. Taken together, these findings provided evidence for a novel mechanism of action for latrepirdine in reducing intracellular accumulation of Aβ42 and attenuation of Aβ oligomer-induced toxicity via activation of autophagy.[51]

Similar observations of latrepirdine-induced autophagy were demonstrated in mammalian models.[21,22] Treatment of cultured mammalian cells with latrepirdine led to enhanced mammalian target of rapamycin- and Atg5-dependent autophagy. Also, latrepirdine treatment of TgCRND8 transgenic mice was associated with improved learning behaviour and with a reduction in accumulation of Aβ42 and α-synuclein. The induction of autophagy was associated with decreased intracellular Aβ accumulation and a trend towards increased secreted Aβ. These changes are consistent with the previous report showing that latrepirdine stimulates secretion of APP metabolites,[72] suggesting that latrepirdine may reduce intracellular APP/Aβ accumulation through stimulation of intracellular catabolic pathways.[22] Latrepirdine was also shown to protect yeast against cytotoxicity associated with α-synuclein expression, and this appeared to

Table 2. Mechanisms of action: latrepirdine

Cellular target/pathway	*Drug activity*	*Reference*
Ion channels and receptor activity	Inhibits voltage-gated calcium channels	5
	Interacts with L-type Ca^{2+} channel, sodium channel, hERG potassium channel and transporter for norepinephrine	17 6,7
	Blocks histamine receptor (H1 and H2) activity	37
	Inhibits NMDA receptors and potentiates activity of AMPA-receptors	17
	Inhibits α-adrenergic receptors (α1A, α1B, α1D, and α2A), imidazoline I2 receptor and serotonin 5-HT2c, 5-HT5A, 5-HT6 receptors	
Mitochondrial activity	Modulate the mitochondrial PTP and suppresses opening of PTP induced by neurotoxins	39
	Attenuates Ca^{2+}-induced mitochondrial swelling	68
	Improves mitochondrial function on aspects such as mitochondrial membrane potential and ATP synthesis	18
	Protects mitochondrial membrane integrity	19
	Enhanced CGU in aged mice	70
	Restore the toxic effects of Aβ on mitochondrial morphology, respiratory chain complex and enlarged mitochondrial mass	69
Protein aggregation	Reduces the number of TDP-43 aggregates in neuroblastoma cells	74
	Reduces the number of amyloid deposits in the spinal cord of over expressing γ-synuclein mice	14
	Reduces accumulation of hippocampal/subicular APP/Aβ and α-synuclein in mice	20–22
	Elevates secretion of Aβ in the extracellular media in neuronal cells and AD transgenic mice	76
	Reduces GFP-Aβ42 in wild-type compared with the autophagy-deficient mutant (Atg8Δ) in yeast model	55
Protein degradation pathways	Upregulates autophagic markers in yeast model	55
	Enhances mTOR- and Atg5-dependent autophagy cultured mammalian cells	22
	Shows increased UPS activity in over expression γ-synuclein mice model	75
Neuroprotective functions	Inhibits MAO deaminating dopamine and serotonin, decrease dopamine metabolism and increase noradrenaline level in the rat brain	46 47
	Inhibits of both acetylcholine esterase and butyrylcholine esterase.	15
	Restores TWAA performance in rats injected with AF64A	5
	Prevents development of convulsions and death caused by NMDA induced toxicity in mice	50
	Reduces amphetamine induced striatal dopamine depletion in mice and promote hippocampus-dependent learning in both appetitive and inhibitory tasks in mice	52 19
	Enhance memory and learning in a primate model	14
	Increases hippocampal neurogenesis in the mouse model	22
	Reduces the development of motor dysfunction in overexpressing synuclein mice model	5,18,55
	Improves spatial memory function and behaviour in AD transgenic mice	
	Protects cultured cells against Aβ toxicity	

Abbreviations: Aβ, β-amyloid; AD, Alzheimer's disease; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; Atg5, autophagy-related protein 5; CGU, cerebral glucose utilization; hERG, human ether-a-go-go-related gene; MAO, monoamine oxidase; mTOR, mammalian target of rapamycin; NMDA, *N*-methyl-D-aspartate PTP, permeability transition pore; TDP-43, TAR DNA-binding protein 43; TWAA, two-way active avoidance; UPS, ubiquitin proteosome.

occur via induction of autophagy. Latrepirdine also stimulated the degradation of α-synuclein in differentiated human neuroblastoma cells, and in mouse brain following chronic administration, in parallel with elevation of the levels of markers autophagic activity.[21] In a more recent study, latrepirdine-treated Thy1mγSN mice showed increased ubiquitin proteosome activity, suggesting that it also stimulates other intracellular systems, in addition to autophagy, that may contribute to the elimination of pathological protein aggregates.[71]

SYNTHESIS AND ACTIVITY OF LATREPIRDINE-RELATED COMPOUNDS

Latrepirdine is a small heterocyclic molecule with different conformations and a relatively complicated pharmacology. As described above latrepirdine can modulate receptor activity, protect mitochondrial function, modulate intracellular catabolic pathways and reduce amyloid aggregates and pathology in cellular and neurodegenerative mice models (Table 2). A wide range of cellular functions and broad concentration ranges (0.1–100 μM) are required to achieve latrepirdine's proposed biological activities. Some of these drug interactions have also been suggested to be important for its cognitive-enhancing functions; however, it has been difficult to identify one particular pathway as a target for latrepirdine in AD and other neurodegenerative diseases.

Latrepirdine is known for its wide array of neuronal functions and hence synthesis of structural analogues and assessment of their effects on the therapeutic targets presents obvious value for developing novel drugs for dementia treatment. In an attempt to isolate potent histamine receptor antagonists, a recent study tested synthesized latrepirdine analogues of 2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indoles 3 and 4 for their ability to interact with histamine H1 receptors.[76] The novel compounds demonstrated much higher affinity to all of the receptors studied as compared with latrepirdine. Steric orientation of 5-styryl substitution was shown to have an important role in the affinity of the molecules to 5-HT6 and H1 receptors. The specificity profiles showed that similar to latrepirdine, the new 2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indoles 3 and 4 display a broad spectra of potential pharmacological activities including adrenergic, dopaminergic, histaminergic, serotonergic as well as some ion channel targets. Although promising, the biological activity of these novel compounds will require further assessment and target validation in cellular and animal models.

Latrepirdine contains a backbone structure of β-carboline (9*H*-pyrido[3,4-*b*]indole), a bioactive class of compounds found naturally in alkaloids produced in plants and known for their

broad spectrum biological activity. The family of β-carboline alkaloids, characterized by a core indole structure and a pyridine ring affect multiple central nervous system targets. These include the 5-hydroxytryptamine receptor subtypes 5-HT2 and 5-HT1A,[77] the NMDA receptor,[78] monoamine oxidase-A[79] and dopaminergic signaling pathways.[80,81] In addition to these targets, the β-carboline alkaloid, harmine, has recently been reported to be a high-affinity inhibitor of dual-specificity tyrosine phosphorylation-regulated kinase 1A activity, a member of the dual-specificity tyrosine phosphorylation-regulated kinase family that has a significant role in signaling pathways regulating cell proliferation involved in brain development.[82] A new class of carboline compound that enhance hippocampal neurogenesis and ameliorate cognitive decline were identified in an *in vivo* screen in search of chemicals capable of enhancing neuron formation in adult mice. In more recent studies, P7C3 has shown the ability to reduce neurodegeneration in ALS and PD models. P7C3 was shown to block MPTP-mediated cell death of dopaminergic neurons in the substantia nigra of adult mice, a model of PD[55] and protect spinal cord motor neurons from cell death in the G93A-SOD1 mutant mouse model of ALS.[57] These observations provide further evidence for the neuroprotective functions of the carboline scaffold and raise the possibility that latrepirdine and P7C3 compounds may operate via a common mechanistic pathway.

CONCLUSION

To date, no preventive or disease-modifying drugs are available for the treatment of neurodegenerative diseases including AD, the leading cause of dementia. Studies have demonstrated neuroprotective functions for latrepirdine and related class of molecules including the β-carbolines and aminopropyl carbazoles. However, the precise mechanism(s) underlying their cognitive benefits in AD, HD and other neurodegenerative disease models is unclear. Further investigation of the specific cellular targets and biochemical pathways underlying their neuroprotective functions in disease models can provide the basis for structure-based drug design. Such target-specific compounds may offer promise for the future in the treatment of neurodegenerative diseases.

CONFLICT OF INTEREST

SG holds research grant support from Amicus Pharmaceuticals, and is a consultant to Pfizer-Janssen Alliance.

ACKNOWLEDGMENTS

We acknowledge financial support from The McCusker Foundation for Alzheimer's disease Research, NHMRC funding (Project Grant APP1009295 to RM, GV and SG) and the Neurotrauma Research Program (KAB). The funding sources had no role in study design, collection, analysis and interpretation of data, the writing of and decision to publish this commentary.

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