Review

Targeting the Cholinergic System to Develop a Novel Therapy for Huntington's Disease

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Abstract. In this review, we outline the role of the cholinergic system in Huntington's disease, and briefly describe the dysfunction of cholinergic transmission, cholinergic neurons, cholinergic receptors and cholinergic survival factors observed in post-mortem human brains and animal models of Huntington's disease. We postulate how the dysfunctional cholinergic system can be targeted to develop novel therapies for Huntington's disease, and discuss the beneficial effects of cholinergic therapies in pre-clinical and clinical studies.

Keywords: Huntington's disease, cholinergic, acetylcholine, choline acetyltransferase, cholinergic receptors, cholinergic drugs

INTRODUCTION

Huntington's disease (HD) is an autosomal dominant, neurodegenerative disorder caused by an extended CAG repeat sequence in the HD gene [1]. Several mechanisms have been proposed to explain how an expanded CAG repeat sequence in the HD gene leads to the symptoms and neuropathology of HD [2–8]. We propose an updated review of the mechanisms through which the cholinergic system could be used to modify the functional deficits and neuropathology of HD.

The cholinergic hypothesis suggests that dysfunctional acetylcholine (ACh)-containing neurons and dysfunctional cholinergic transmission in the brain significantly contribute to the behavioural symptoms and neuropathology in disease, in this instance HD [9]. The link between the cholinergic hypothesis and neurodegenerative disease was proposed by Bartus and colleagues in a seminal review [10] which referred to findings from previous studies including the cognitive deficits produced by anticholinergic drugs in humans [11, 12] and primates [13], as well as reduced choline acetyltransferase (ChAT) activity, reduced ACh release and degeneration of cholinergic neurons in autopsied patients [14–20].

The electrophysiological properties of cholinergic neurons vary depending on their location in the brain. Cholinergic neurons in the ventral pallidum and magnocellular cholinergic neurons in other parts of the forebrain, identified by ChAT staining, displayed a large whole cell conductance, a hyperpolarized resting membrane potential, marked fast inward rectification, a prominent spike afterhyperpolarization (AHP), but did not fire spontaneously [21]. However,

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these findings were based on a single study in rat brain slices [21].

In addition cholinergic neurons in the basal fore-brain of GFP-expressing transgenic mice can be either early-firing or late-firing neurons [22]. The early-firing neurons are more excitable and are more susceptible to depolarization blockade, while displaying prominent spike frequency adaptation. Conversely, late firing neurons are less excitable and maintain a tonic discharge at low frequencies. Early-firing neurons are thought to be involved in phasic changes in cortical ACh release associated with attention, while the late-firing neurons may support general arousal by maintaining tonic ACh levels [22].

The distinct electrophysiological properties of cholinergic neurons means that it would be possible to distinguish cholinergic neurons from non-cholinergic neurons in brain slices, which will simplify future physiological and pharmacological studies of these neurons. The existence of two distinct subtypes of basal forebrain cholinergic neurons and their electro-physiological properties suggests that each subtype has a different ACh release profile, which is supported by recent studies showing ACh release can be mea-sured over seconds or minutes [23–25]. Further, it is thought that each subtype is involved in different aspects of synaptic plasticity [22].

The afferent inputs and efferent outputs of cholin-ergic neurons also vary according to their location. Muscarinic cholinoceptive neurons in the neocortex are directly innervated by the magnocellular basal nucleus, while basal forebrain cholinergic neurons receive afferent input from the prefrontal cortex [26]. Cholinergic neurons from the basal forebrain and upper brainstem project to several areas including the cerebral cortex, amygdala, hippocampus, olfac-tory bulb and the thalamic nuclei [27]. The amygdala and pyriform cortex also receives cholinergic projec-tions from the substantia innominata [28]. In addition cholinergic neurons in and around the pedunculo-pontine nucleus were shown to send projections to the substantia nigra pars compacta (SNc) via nico-tinic receptors [29]. The substantia nigra also receives innervation from cholinergic cells of the rostral pon-tine tegmentum [28].

Striatal cholinergic interneurons densely innervate the striatum resulting in the striatum having one of the highest levels of ACh in the brain. These neurons, known as tonically active neurons (TANs) of the stria-tum, function as pacemaker cells and exhibit single spiking or rhythmic discharges but fire autonomously at rest. They receive dopaminergic input from the SNc, glutamatergic input from the thalamus (from the intralaminar nuclei) and the cerebral cortex, as well as input from the brain stem. In addition they contact each other. TANS modulate other interneu-rons particularly the fast spiking parvalbumin positive GABAergic interneurons and most importantly the medium spiny neurons (MSNs) through complex synaptic interactions [30]. They project to virtually all MSN subtypes, including both D_1- and D_2-dopamine receptor expressing MSNs and directly act through M_1 receptors located on MSNs [31, 32]. The combined activation of the A_{2A} adenosine and D_2 dopamine receptors on cholinergic interneurons decreases the release of ACh. This decrease reverses the blockade of L-type calcium channels, which are mediated by M_1 muscarinic receptors located on MSNs, which triggers the entry of calcium into the MSN. This might in turn trigger endocannabinoid release at the postsynaptic sites of both D_1- and D_{2R}-expressing MSNs and thus depress the corti-costriatal and thalamostriatal glutamatergic synaptic transmission of both the direct and indirect pathways [31, 32].

Mouse models of HD have shown anatomical changes of striatal cholinergic neurons and reduced thalamic input to striatal cholinergic interneurons [33]. Since cholinergic interneurons have different effects on the striatal spiny projection neurons of the direct and indirect pathways, the reduction of glutamatergic transmission from corticostriatal and thalamostriatal projections may be responsible for early motor impairments observed in HD [33].

EVIDENCE FOR THE CHOLINERGIC HYPOTHESIS IN HUNTINGTON'S DISEASE

Studies in post-mortem human HD brains and animal models have shown that dysfunctional cholin-ergic neurotransmission and cholinergic neurons may play a significant role in HD pathogenesis. Post-mortem brains of patients with HD have shown a reduction in the levels of ChAT, vesicular acetyl-choline transporter (VAChT), vesicular monoamine transporter type-2 (VMAT2) and the pyruvate dehy-drogenase complex, an essential enzyme for ACh synthesis [34–39]. Conversely, choline levels were down in the CSF of HD patients but there was no reduction in the levels of acetylcholinesterase (AChE), an enzyme which degrades ACh [40]. Impairments in cholinergic neurotransmission were

also observed in animal models of HD. Young R6/1 transgenic mice exhibited reductions in AChE activity and ChAT and VAChT messenger ribonucleic acid (mRNA) and protein levels [41, 15]. Transgenic R6/2 mice also showed a significant reduction in the electrically-evoked release of ACh in striatal tissue, as well as a decrease in ChAT activity and synaptosomal high-affinity choline uptake with age [42, 43].

In HD, striatal GABAergic MSNs are preferentially degraded, while large aspiny cholinergic interneurons are relatively spared [44, 45]. However, the surviving striatal cholinergic neurons showed decreased ACh release, VAChT binding and significant reductions in ChAT activity, but persistent AChE activity [34, 45–47]. During the early and mid-stages of HD, these cholinergic neurons demonstrate neuronal dysfunction without cell death, which may be involved in the early symptomatology of HD [41, 48]. The dysfunction observed in striatal cholinergic neurons may impact ACh-dependent processes such as the induction of synaptic plasticity [43, 47], and may significantly affect the function of other neuronal populations, including MSNs [48–50]. However, in the later stages of HD, the loss of aspiny cholinergic neurons has been observed [51–53].

Studies carried out in post-mortem human HD brains have produced varying results. Some have shown a reduction in muscarinic acetylcholine receptor (mAChR) binding, and a decrease in the number of M_2 mAChRs and total mAChRs [35, 37]. Conversely, early studies reported no change in the levels of nAChRs in post-mortem human HD brains [54, 55]. However, these studies also reported no alterations in the level of ChAT in human HD brains, which has since been refuted by subsequent studies, and further research is therefore needed to clarify the levels of nAChRs in the HD brain [34–37]. Muscarinic cholinergic receptors were also decreased in R6/2 mice [56].

Decreased levels of NGF, an essential regulator of cholinergic neuronal survival and cholinergic neurotransmission, were observed in HD patients and R6/1 mice [48, 57]. Altered levels of NGF receptors have also been reported in the hippocampus of HD patients and in R6/1 and Hdh$^{Q7/Q111}$ mice [58, 59].

CHOLINERGIC TREATMENTS FOR HUNTINGTON'S DISEASE

The first procholinergic treatment for HD was the ACh precursor, choline, which significantly reduced chorea in three out of eight patients after they were administered choline (Table 1) [60]. However, choline showed no beneficial effects in other clinical studies [61, 62].

Another therapeutic strategy was to increase ACh levels through the use of acetylcholinesterase inhibitors (AChEIs) which inhibited the breakdown of ACh by the enzyme AChE (Fig. 1). The AChEI, rivastigmine, enhanced cognitive performance and slowed motor deterioration in an open-label clinical trial of 21 HD patients [63]. In a follow-up study, patients treated with rivastigmine showed increased motor performance, reduced chorea, increased cognitive function and reduced functional disability, relative to controls, suggesting that rivastigmine may have long-term benefits as a potential treatment for HD [64]. A smaller clinical study on four HD patients reported that rivastigmine improved cognitive and behavioural function, but failed to improve motor function or functional performance (Table 1) [65]. The AChEI, donepezil, improved the cognitive function of R6/2 mice in a simple visual discrimination task (Table 2) [66]. However, the beneficial effects of donepezil did not translate to the clinic [67, 68]. Galantamine, another AChEI, attenuated neurological deficits, reduced striatal lesion volumes and decreased the number of apoptotic cells in the 3-nitropropionic acid rat model of HD [69]. Further, a 35 year old male patient treated with galantamine displayed an improvement of psychotic symptoms and chorea, but showed no change in cognitive function [70]. Interestingly, the beneficial effects of galantamine were attenuated by mecamylamine, a nAChR antagonist, which suggests that the beneficial effects of galantamine may be mediated through nAChR activation [69].

AChEIs can also activate the cholinergic anti-inflammatory pathway, which reduces the neuroinflammation associated with HD pathology [71, 72]. These anti-inflammatory effects were inhibited by the α7 nAChR antagonist MLA, but not by the α4β2 nAChR antagonist, dihydro-β-erythroidine, which suggests that the anti-inflammatory effects of the cholinergic anti-inflammatory pathway are mediated through α7 nAChRs [73–76]. Physostigmine, an AChEI which stimulates both nAChRs and mAChRs, was shown to reduce chorea in two studies [77, 78]. Muscarinic receptor activation was also shown to reverse impairments in spatial memory, enhance hippocampal cell proliferation in the dentate gyrus and the CA1 region of the hippocampus and reverse deficits in hippocampal neurogenesis (Fig. 1) [79].

Table 1
Cholinergic-based treatments which have demonstrated beneficial effects in clinical HD studies. Strength of evidence criteria was based on proper study design, number of subjects improved, level of improvement, lack of side effects and replication by others

Treatment	Beneficial effect(s)	Number of subjects studied	Reference(s)	Strength of evidence	Comments
Choline	reduces chorea	8	[60]	3/10	3 out of 8 showed reduced chorea.
Rivastigmine	improves motor and cognitive function, reduces chorea	21 patients, 17 patients, 4 patients	[63–65]	8/10	Beneficial cognitive and motor effects in a trial and then again in a follow-up study. Beneficial cognitive effects replicated in separate study. Study design could be improved.
Galantamine	improves psychotic symptoms and chorea	1 patient	[70]	3/10	Positive effects, but only 1 patient.
Physostigmine	reduces chorea	4 patients; 3 patients	[77, 78]	4/10	Reduction in chorea seen in both studies, but low number of patients studied.
Scopolamine	reduces chorea	4 patients	[80]	2/10	Reduction in chorea, but side-effects include incoordination, sedation and confusion.
Benztropine	reduces chorea	4 patients	[80]	2/10	Similar but milder effects compared to scopolamine
Olanzapine	improves motor and psychiatric function	9 patients; 11 patients; 1 patient; 2 patients	[81–84]	5/10	Beneficial effects were replicated across studies.

An alternative therapeutic strategy was to use anticholinergic agents, such as muscarinic receptor antagonists, to reduce chorea since anticholinergics cause sedation. The mAChR antagonist, scopolamine, decreased chorea but increased lack of coordination, sedation and confusion [80]. Another mAChR antagonist, benztropine, produced similar but milder effects, while the peripheral anticholinergic agent, glycopyrrolate, had no effect on chorea [80]. Conversely, the M_1 mAChR antagonist, olanzapine, improved motor function [81] and psychiatric function [82] in human HD patients. Other studies reported that olanzapine improved both motor and psychiatric function when administered alone [83] or together with valproate [84]. The M_4 mAChR positive allosteric modulator VU0467154 was reported to improve motor function and reduce synaptic deficits in transgenic YAC128 mice [85]. Further, the depotentiation of spiny neurons was blocked by either scopolamine or hemicholinium in mouse and rat models, which suggests that the reversal of LTP requires the activation of muscarinic receptors [86].

The administration of NGF and NGF-promoting drugs, cells or microspheres, increased motor coordination [87], reduced clasping [87], increased spatial working memory [48, 88], showed neuroprotective effects [89–94], reversed cholinergic neuron atrophy [88], promoted hippocampal neurogenesis [48], and increased the expression of ChAT and VAChT [48, 95] in rodent models of HD (Table 2).

There are therefore several potential targets for therapeutic intervention in the cholinergic system including (1) increasing the amount of the cholinergic survival factor NGF (2) increasing the amount of the ACh precursor choline (3) using AChEIs which inhibit AChEs from breaking down ACh and (4) using mAChR/nAChR ligands to activate mAChRs and nAChRs. Of these, the most promising therapeutic intervention appears to be increasing NGF, because if NGF can restore cholinergic neurons to optimal function and enhance ACh release to enable proper MSN function, there is no need to artificially add choline, AChEIs or mAChR/nAChR ligands to the system.

CONCLUDING REMARKS

Studies showing the dysfunction of cholinergic transmission, cholinergic receptors, cholinergic neurons and their associated neurotrophic factors in human HD patients and animal models of HD, together with the close link between cholinergic
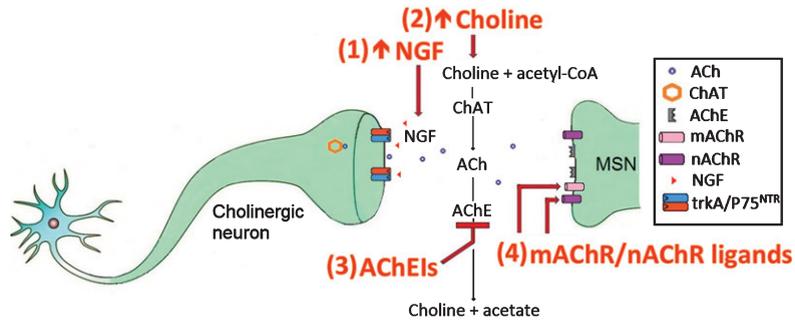
Fig. 1. The cholinergic system in HD and targets for therapeutic intervention.

neurons and MSNs, suggest that the dysfunctional cholinergic system plays a vital role in the symptoms and neuropathology of HD. This provides researchers with a rationale to develop potential therapies for HD by targeting the cholinergic system. Cholinergic treatments have shown beneficial effects in both pre-clinical and clinical studies, and have improved motor, cognitive and psychiatric function, as well as reducing striatal lesions, synaptic deficits, excitotoxicity, cell atrophy and cell death [63, 64, 69, 85, 88]. However, different studies often reported different efficacies for the same therapy, possibly due to differences in the model or criteria used to determine efficacy, dose administration technique and dosing regimen used [79]. In order to achieve optimal results, these factors need to be optimised. In addition, a potential cholinergic therapeutic should be administered during the stage of the disease where it can have the greatest effect, for example, a mAChR agonist should be administered before significant loss of mAChRs occurs. Nonetheless, we believe that the beneficial effects of cholinergic treatments in pre-clinical and clinical HD studies demonstrates that this is a promising avenue of HD research which requires further investigation, and that enhancing cholinergic function may lead to the development of a potential therapy for HD.

The interdependent nature of the cholinergic dysfunction and its associated neurotrophic factors means that a potential treatment targeted towards increasing cholinergic neurotrophic factors may also have beneficial effects on cholinergic neuronal survival and neurotransmission [48]. In addition, the widespread effects of the cholinergic system on brain function suggests that treatments targeted towards the normalization of cholinergic transmission in HD may have other unseen beneficial effects, such as improving the function of other neuronal populations, such as MSNs [48, 50, 96].

Based on the available data (see Table 1), one of the most-promising therapeutic avenues appears to be increasing the levels of NGF, either through direct administration, the administration of NGF-promoting drugs, such as riluzole, or NGF-secreting cells or microspheres. NGF is an important survival factor for cholinergic neurons, and thus mediates cholinergic transmission as well as the function of neurons innervated by cholinergic neurons, such as MSNs. To investigate the pre-clinical efficacy of NGF, an animal model that replicates the human condition as closely as possible such as the YAC128 transgenic mouse model of HD, could be used. This mouse contains the full-length human HD gene and exhibits the motor, cognitive and psychiatric symptoms of HD, as well as the neuropathological characteristics, such as striatal cell loss, huntingtin aggregates and altered synaptic currents [97, 98]. NGF does not cross the blood-brain-barrier [99], thus, we would deliver NGF by surgically implanting microspheres in the striatum of the mice to achieve a controlled and sustained delivery of NGF [91]. The mice would undergo behavioural testing, and various neuropathological endpoints including MSN and cholinergic cell survival as well as synaptic function [48, 85, 100, 101]. Since therapies geared towards a single target have failed to show beneficial effects in the clinic, a therapy, such as NGF, which can affect multiple targets, may have a higher chance of exhibiting beneficial effects in a clinical trial [102].

Recent studies have focused on specific cholinergic receptor subtype agonists and antagonists, in particular those targeting $\alpha4\beta2$ and $\alpha7$ nicotinic acetylcholine receptors (nAChRs). The selective $\alpha4\beta2$ nAChR agonist SIB-1508Y exerted anti-Parkinsonian effects in monkeys [103]. The selective $\alpha4\beta2$ agonists ZY-1 and TC-6683 increased cognitive function in preclinical animal models of

Table 2
Cholinergic-based treatments which have demonstrated beneficial effects in pre-clinical HD studies. Strength of evidence criteria was based on proper study design, number of subjects improved, level of improvement, lack of side effects and replication by others

Treatment	Beneficial effect(s)	Animal model	Number of animals studied	Reference(s)	Strength of evidence	Comments
Donepezil	improves cognitive function	R6/2 transgenic mice	First study: $n = 73$ (across 4 groups); second study: $n = 55$ (across 4 groups)	[66]	8/10	Appropriate study design, appropriate mouse model, high number of mice used, results replicated in individual studies.
Galantamine	reduces neurological deficits, striatal lesions and apoptosis	3-nitropropionic acid rat model	$n = 46$ (across 5 groups)	[69]	4/10	Appropriate study design although some groups with low numbers of mice, animal model used not ideal.
VU0467154	improves motor and synaptic function	YAC128 transgenic mice	Motor function: $n = 41$ (across 4 groups); synaptic function: $n = 43$ (across 4 groups)	[85]	6/10	Appropriate study design, highly valid animal model used.
Physostigmine	increases hippocampal cell proliferation	Wistar rats	$n = 30$ (across 5 groups)	[79]	4/10	Animal model not HD-specific, relatively low numbers of animals.
Oxotremorine	increases hippocampal cell proliferation, spatial working memory and reverses deficits in hippocampal neurogenesis	Wistar rats	$n = 30$ (across 5 groups)	[79]	5/10	Multiple beneficial effects but animal model not HD-specific.
NGF	improves motor and cognitive function; increases cholinergic cell survival, expression of ChAT, VAChT and trkA markers, hippocampal neurogenesis; reduces excitotoxicity and cholinergic cell atrophy	R6/1 mice, YAC128 mice, Sprague-Dawley rats, Wistar rats	R6/1 mice: $n = 48$ (across 4 groups); YAC128 mice: $n = 64$ (across 4 groups); SD rats study 1: $n = 29$ (across 4 groups); SD rats study 2: $n = 27$ (across 4 groups); SD rats study 3: $n = 37$ (across 4 groups); SD rats study 4: $n = 18$ (across 3 groups) Wistar rats: $n = 8$ (across 2 groups)	[48, 87, 88, 90–95]	9/10	Appropriate study designs, valid animal models used, numerous beneficial effects reported, beneficial effects found by multiple research groups.

AD, with TC-6683 having progressed to phase 2 clinical trials for AD [104, 105]. The selective α4β2 agonist altinicline, and partial α4β2 agonists cytisine and ispronicline displayed antidepressant-like effects [106, 107, 108]. The α7 nAChR agonist, PNU-282987, improved the motor coordination of rats [109]. Further, administration of EVP-6124, an α7 nAChR partial agonist, and TC-5619, a full α7 nAChR agonist produced improvements in the memory of rats [110, 111].

Given the beneficial effects of α4β2 and α7 nAChR agonists on motor, cognitive and psychiatric function, it may be beneficial to pursue a potential therapy which activates both α4β2 and α7 nAChRs. One such drug is varenicline, a potent, partial agonist at α4β2 nAChRs, and a potent, full agonist at α7 nAChRs [112, 113]. Varenicline increased basal locomotor activity in rats [114]. Varenicline also enhanced the cognitive function of mice [115, 116] and rats [117]. Further, varenicline showed antidepressant-like activity [118], and anxiolytic effects [119].

The potentially advantageous therapies described above which directly or indirectly target the cholinergic system may benefit HD patients, and should therefore be further investigated.

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