Virtues and woes of AChE alternative splicing in stress-related neuropathologies

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The ACh hydrolyzing enzyme acetylcholinesterase (AChE) is a combinatorial series of proteins with variant N and C termini generated from alternate promoter usage and 3' alternative splicing. Neuronal AChE variants show indistinguishable enzymatic activity yet differ in their expression, multimeric assembly and membrane-association patterns. Differentially induced under stress, they show distinct non-hydrolytic properties and interact with different protein partners. Recent findings suggest that transcriptional and post-transcriptional regulation of AChE pre-mRNA is a neuroprotection strategy but might involve long-term damage. Specifically, variant-specific causal involvement of AChE in the progression of both neurodegenerative diseases (e.g. Alzheimer's and Parkinson's diseases) and neuromuscular syndromes (e.g. myasthenia gravis) raises the possibility that future therapeutic drugs might target specific AChE variant(s) or the corresponding RNA transcripts.

The composition and role of AChE

Much has been learned and said about both the hydrolytic and the non-catalytic roles of acetylcholinesterase (AChE) [1–3]. Its primary role undoubtedly remains that of hydrolyzing synaptic ACh. In this role, AChE is one of the most efficient enzymes in nature, capable of hydrolyzing ACh at a rate so high that it is limited only by diffusion [4]. This property makes it evolutionarily an almost perfect terminator of ACh-mediated neurotransmission. The discovery that AChE knockout mice were born alive was thus unexpected, especially because AChE is required for neuronal and muscular development in zebrafish embryos [5]. Despite their delayed physical development, the Ache−/− mice lived for up to 21 days and displayed apparently normal neuromuscular junctions (NMJs) [6]. Their survival is most probably due to partial functional redundancy with other catalytic enzymes, such as butyrylcholinesterase, that can partly compensate for some AChE functions [7].

The properties of AChE are modified during many CNS neuropathologies; this impedes the intricate balance between neuronal stimulation and termination, and alters synaptic properties and neurotransmission. The properties of AChE also vary in non-pathological situations, and three alternatively spliced isoforms of the enzyme have been identified. The canonical tetrameric AChE-S isoform (‘S’ indicates synaptic; this isoform is also known as AChE-T, with ‘T’ indicating tailed) is produced by joining exon 4 with exon 6 to give a cysteine-containing, and thus tetramerizing, C terminus (Figure 1a). An additional 3' spliced form of AChE is generated by missing out the short intron 4, to join exon 4 directly with exon 5. This gives rise to the dimeric glycosphatidylinositol (GPI)-anchored blood-expressed AChE-E isoform (‘E’ indicating erythrocytic; this isoform is also known as AChE-H, with ‘H’ indicating hydrophobic). Additional 3' alternative splicing also generates the ‘read-through’ variant of AChE (AChE-R) [8,9]. Although AChE-R is found predominantly under conditions of neurodegenerative disease and stress-associated disorders, it also occurs to a minor extent in healthy tissue and myogenic cell lines [10]. AChE-R is produced by retaining intron 4 and exon 5, to yield an AChE mRNA variant that has an alternative 3' end (Figure 1a). This transcript generates an alternative protein product that has a distinct shorter C terminus and lacks the cysteine residue present in the C terminus of AChE-S; AChE-R thus lacks multimerization potential and remains monomeric and soluble [1,11]. Therefore, AChE can be monomeric (AChE-R), dimeric (AChE-E) or tetrameric (AChE-S). Tetrameric AChE-S can further interact with collagen Q (ColQ), enabling anchorage to NMJs [11], and a proline-rich membrane anchor protein (PRiMA) is responsible for the synaptic docking of AChE-S in the brain [12]. Because AChE-E and AChE-R are incapable of anchorage to the NMJ or to synaptic membranes through ColQ or PRiMA, only the AChE-S form of the enzyme is regarded as truly ‘synaptic’. The 3' alternatively spliced transcripts described so far are common to all mammals and are ubiquitously expressed. However, an examination of the expression sequence tag (EST) database reveals that a novel 3' alternatively spliced variant has been repeatedly observed exclusively in pooled germ-cell tumors of human origin (GenBank accession numbers BE502241,
AW590331, AI825097, AI632562, AI341526, AI205484, AW138046, BE504052, AI651502 and BE502360). This variant, designated AChE-E

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generated by an exclusion of a cryptic intron within exon 5, yielding a transcript 36 bases shorter than the canonical AChE-E (Figure 1a,ii; Figure 1c,ii).

In addition to the 3′ alternative splicing of AChE that generates proteins with distinct C termini, the 5′ end is

also subject to intricate regulation, with at least five alternatively spliced and differentially expressed exons in mouse and three in human [13] (Figure 1a,b). Interestingly, each of these transcripts is associated with a distinct promoter region at its 5′ end, and some are developmentally regulated and/or responsive to stress [13]. Sequence conservation between mouse Ache and human ACHE is also evident at the 5′ end, especially surrounding the

\( AChE-S \)
\( AChE-R \)
\( AChE-E \)

Figure 1. AChE gene structure, alternative transcripts and protein products. (a) Structure of the mouse Ache (i) and human ACHE (ii) genes (drawn to the same scale). Exons are depicted as cylinders, introns as horizontal lines. Splicing options are shown as lines above the genes. E1a–1e are alternative versions of mouse Ache exon 1, and hE1a, hE1c and hE1e are alternative versions of human ACHE exon 1. 4′ indicates the pseudo-intron 4 (shown in red); 5′ indicates the cryptic intron skipped in germ-cell tumors. Abbreviation: GRE, glucocorticoid response element. (b) Homology in the 3-kb-upstream regions of mouse Ache (i) and human ACHE (ii). Parallel homologies are shown in the same shades of gray, and alternative 5′ exons are shown underneath in the same colors as in (a). (c) Combinatorial complexity of mouse Ache (i) and human ACHE (ii) mRNA transcripts. Each of the five murine alternative 5′ first exons can potentially be combined with the three murine 3′ alternative transcripts, and each of the three human alternative 5′ exons can potentially be combined with the four human 3′ alternative transcripts, to yield 15 or 12 alternatively spliced isoforms, respectively. (d) AChE alternative protein products. (i) Globular structure of tetrameric AChE-S (green), monomeric AChE-R (red) and dimeric AChE-E (gray). (ii) Conventional wisdom regarding the membrane attachment of AChE isoforms. Synaptic docking of AChE-S is mediated by ColQ or PRiMA (black) and that of AChE-E is mediated by a GPI anchor (purple). AChE-R remains monomeric. (iii) Putative membrane docking through the extended N terminus (N-AChE, yellow) transcribed by the alternative exon E1e of AChE-S (green), AChE-R (red) or AChE-E (gray).
alternative 5′ exons (Figure 1b). The different 5′ versus 3′ alternative combinations can potentially give rise to 15 and 12 different AChE transcripts in mice and humans, respectively (Figure 1c).

The only hydrophobic sequence in the canonical AChE protein is its first 31 amino acids. These primarily constitute a signal peptide that docks AChE to the endoplasmic reticulum membrane and is later cleaved off during maturation. In the three AChE isoforms with distinct C termini already described, this leader sequence is removed [14]. Intriguingly, although most of the recently identified 5′-encoded exons produce alternative untranslated regions (UTRs), at least one of those exons gives rise to a protein that has an extended N-terminus in humans, designated N-AChE [13]. In principle, N-AChE-S, N-AChE-E and N-AChE-R (or various combinations thereof) might exist (Figure 1d). Because N-AChE associates with the plasma membrane in human blood cells [13], it is tempting to speculate that the extension prevents cleavage of the signal peptide, as is the case for cyclooxygenase [15]. This extension could then serve as a transmembrane domain, enabling AChE to anchor itself to the synaptic membrane (Figure 1d). The existence of N-AChE could have profound neurological implications, especially in relation to the expression of monomeric AChE-R: the N-terminal extension might provide a means by which AChE-R can dock itself — without the assistance of CoQ or PRIMA — to the synaptic membrane.

AChE in stress-related neurological disorders
Transcriptional and post-transcriptional stress-related responses interact to induce a cascade of changes in the levels and properties of brain proteins that might be involved in neuronal reactions to stimuli [16]. Proteins involved in stress-related neurotransmission pathways are particularly affected in this way. ACh levels are transiently elevated in the mammalian brain during stress responses [17], and similar increases in ACh-mediated signaling have been reported following exposure to cholinesterase inhibitors [18]. Indeed, psychological stress elicits strikingly similar neuropsychological effects to those observed after acute or chronic exposure to anticholinesterases [19]. In addition, acute stress and pharmacological inhibition of AChE both suppress the production of the ACh-synthesizing enzyme choline acetyltransferase (ChAT) [8]; such suppression is accompanied by rapid increases in ACh-E-R mRNA levels in the mouse brain in response to forced-swim stress [8,9].

Several lines of evidence suggest that AChE-S and AChE-R have distinct, and sometimes inverse, roles in the normal brain and in post-stress processes [20–22]. Transgenic TgS and TgR mice overexpressing AChE-S or AChE-R, respectively, display distinct characteristics [1]. TgS mice show accelerated stress-related neuropathology [20], including loss of dendritic arborizations and spines. This leads to progressive deterioration, neuromotor malfunctions, hypersensitivity to anti-cholinesterases and to closed-head injury, vulnerability to a switched day–night cycle, and impaired diffusion across the blood–brain barrier [1,22,23]. AChE-R-expressing TgR mice, by contrast, display normal neuromuscular function and their brains are relatively protected from the stress-associated pathology hallmarks that predict age-dependent neurodegeneration [20] (e.g. astrocyclic hypertrophy). Stress-induced alternative splicing of AChE might be a cause or an outcome of this phenotype; in either case, it could predict physiological relevance for the exclusive overexpression of AChE-R following stress. Apart from this apparent protective function, AChE-R might mediate at least some of the adverse cellular changes associated with delayed stress responses. It has recently been shown that the stress-induced increase in AChE-R mRNA yields a marginal subsequent increase in the corresponding enzyme activity in many brain regions [24], suggesting that much of the newly synthesized protein is catalytically inactive. However, even a low-level increase in soluble AChE can have a profound effect, especially on non-cholinergic neurons carrying ACh receptors. Altogether, this response suggests a bimodal potential mechanism for suppressing ACh-mediated excitation after stress. Intriguingly, ACh-E-R mRNA was found in apical dendrites of neurons from all cortical layers following exposure to cholinesterase inhibitors [8], suggesting possible local regulation and translation of AChE in the synapses of non-cholinergic neurons. The robust and persistent post-stress AChE-R overexpression [8,9] is further accompanied by translocation of ACh-E-R mRNA to neurites following repeated stress in mice. There, it replaces the usually prevalent AChE-S mRNA [9]. This stress-induced shift from AChE-S to ACh-E-R mRNA is strongly associated with synaptic hypersensitivity to anticholinesterases (Figure 2). When physostigmine was applied to brain sections of mice weeks after a period of repeated stress, a dramatic increase in extracellular population field potentials (PFPs) was observed in response to stratum oriens stimulation [9]. Atropine, a selective and reversible competitive antagonist of muscarinic acetylcholine receptors (mACHRs), reduced the recorded electrophysiological activity in pre-stressed synapses to almost zero. This might suggest that a negative-feedback loop operates during stress to down-regulate the levels of postsynaptic mACHRs. Inhibition of a reduced number of receptors would reduce synaptic activity (Figure 2b). Supporting this notion, stress-induced reduction in mACHRs was reported in a rat model of Gulf War syndrome [25]. Interestingly, the evoked PFP was blocked by the NMDA receptor antagonist aminophosphonopentanoic acid (APV) and the AMPA receptor antagonist dinitroquinoxalinedione (DNQX), attesting to the glutamate-mediated (i.e. excitatory) nature of the hypersensitized synapses. This phenomenon closely resembles the hypersensitivity that has long been known to follow stressful experiences [26], suggesting involvement of AChE in stress-related symptoms and deleterious functional consequences of AChE-R overexpression.

Because stress-induced neuronal hypersensitivity occurs through intensification of ACh-induced signaling cascades [9] (Box 1, Figure 3b), it might be mediated by interaction of intracellular AChE-R with the protein kinase C (PKC)-scaffold protein RACK1 [27]. PKC mobilization to the perikaryal membrane [27] and the
intensification of long-term potentiation (LTP) in AChE-R-overexpressing brain [28] could provide a new explanation for the well-known phenomenon of sensitization following stress [26,29]. That antisense suppression of AChE-R accumulation obliterates the consolidation of fear memories [28] corroborates this concept. Interactions between ACh and glutamate signaling have been demonstrated in several brain regions (Box 1) to affect CNS functions such as LTP, memory and behavior [30], all of which are subject to hyperexcitation following stress. The glutamate-mediated nature of the hypersensitized response might further be relevant to the additional functions attributed to neuritic AChE-R. In particular, based on the electrotactin properties of AChE [2], non-catalytic capacities might compete with, and mediate, cell–cell and cell–matrix interactions. Neuroligin-1, for example, is a postsynaptic cell-adhesion molecule in excitatory synapses [31] that includes an extracellular catalytically inactive AChE-homologous domain [1]. When overexpressed, AChE-R might compete with neuroligin-1 for its partner protein β-neurexin [32]. However, the distinct Ca$^{2+}$-binding domains in AChE and neuroligin-1 [1] predict differences in their adherence properties, suggesting that excess AChE-R might modify glutamate-mediated functions by impairing neurexin–neuroligin interactions. Non-hydrolytic roles of AChE have recently been challenged [3], suggesting that all previously reported phenotypes can also be explained by the catalytic actions of AChE on ACh. However, in a recent study, the similar effects of both active AChE-S (TgS) and inactive AChE-S (TgSm) transgenes on the proliferation of neural

**Box 1. Interactions of the cholinergic and glutamatergic systems**

The functional synergetic interaction between glutamate and ACh neurotransmission was initially demonstrated >12 years ago, by a study in which antagonists of glutamate and ACh receptors impaired performance on a delayed non-matching-to-sample task when given jointly, but not when each drug was administered alone [63]. The activation of NMDA receptors reduced ACh release, a mechanism that places local ACh release under the control of glutamatergic afferents [64]. Additional studies established the ACh–glutamate connection with regard to cerebral blood flow [65], cognition [66] and memory [67,68], using different combinations of specific inhibitors. More recently, using both immunostaining and electrophysiology, glutamate-mediated expression and regulation have also been demonstrated at the frog NMJ, a synapse innervated by a single cholinergic neuron [69]. In another model, metabotropic glutamate receptors have been shown to be expressed in rat striatal cholinergic interneurons [70], and their modulatory functions have also recently been demonstrated [71]. In cultured hippocampal neurons, excessive expression of AChE impairs glutamate-mediated synaptogenesis [72]. Interestingly, in this study, chronic inhibition of the peripheral anionic site of AChE – thought to mediate non-enzymatic roles through protein–protein interactions – impaired glutamate-mediated functions independently of cholinergic neuron activation. Finally, a transgenic model of chronic overexpression of synaptic AChE (TgS mice) showed upregulation of glutamate receptors in the prefrontal cortex (PFC) compared with control mice [22]. The capacity of the PFC to regulate ACh release in the posterior parietal cortex via glutamate-mediated and ACh-mediated prefrontal mechanisms has recently been demonstrated [73], establishing interactions of the cholinergic and glutamatergic systems in the stress-prone PFC. Inhibition of glutamate-mediated neurotransmission abrogated the hypersensitivity of ACh-mediated transmission to physostigmine induced by repetitive stress regimes [9], attesting to the interactions between ACh and glutamate activity in electrical signaling after stress.
progenitors in the developing murine brain suggests otherwise [33].

Although our focus here is on the CNS, stress-induced AChE-R overexpression is not limited to the brain. AChE-R mRNA transcript accumulates in many tissues during embryonic development, and after exposure to different stressors of intestinal epithelium cells, blood-cell progenitors in the developing murine brain suggests that the capacity to overproduce AChE-R in reaction to various stressors is an important determinant of ability to cope with anxious stimuli. In blood, AChE-R is rapidly inactivated [38], but it might be protected from both oxidative stress and inhibitory anti-cholinesterases by PON1 [39]. This explains why debilitating PON1 polymorphisms, which increase the risks of elevated state anxiety [37], also increase the risk of developing pesticide-induced Parkinsonism [40]. The freedom to overproduce AChE-R when needed thus mediates interactions of ACh signaling with both the glutamatergic and dopaminergic systems.

**AChE variants in Alzheimer’s disease, Parkinson’s disease and myasthenia gravis**

Alzheimer’s disease (AD), Parkinson’s disease (PD) and myasthenia gravis (MG) are the three best-studied neuropathologies associated with AChE alterations. AD is the most prevalent type of dementia in the elderly and is characterized by deposition of β-amyloid protein, which is processed by proteolytic cleavage of the β-amyloid protein precursor [41]. Forming of these deposits or plaques is characteristic of the early pathogenesis in AD. Cholinergic circuits are supposedly not impaired until later stages of AD [42]; nevertheless, recent evidence strongly suggests that AChE contributes to early plaque formation and pathogenesis [43]. This might suggest non-hydrolytic role(s) for AChE in the progression of AD. Most of the currently approved drugs for AD are anti-cholinesterases, which bind the active site of AChE and inhibit its action. However, active-site inhibitors might not affect the non-catalytic actions of AChE, which possibly partake in disease progression and severity. In addition, modified alternative splicing of AChE pre-mRNA induces AChE-R excess in AD [44]. Long-term intake of AChE inhibitors by individuals with AD selectively exacerbates these isoform-specific changes. Rivastigmine, for example, causes a selective increase in levels of AChE-R but not AChE-S [44]. Targeting a specific AChE isoform (e.g. by antisense or siRNA agents) could hence offer specific advantages. Alternative splicing of other transcripts has also been reported in AD. For example, a hypoxia-inducible form of presenilin 2 (PSEN2), which is generated by alternative splicing, is prevalent in the brains of individuals with AD accumulation in various cells and tissues might thus contribute to a complex physiological outcome.

Stress can induce an overwhelming feeling of fear; when that fear is out of proportion to its origin, it is defined as anxiety [34]. Anxiety-related disorders are the most common psychiatric disorders and are closely related both to stress [35] and to stimulation of cholinergic neurons [36]. A genetic study into a potential correlation between polymorphisms in the AChE–paraoxonase 1 (PON1) locus and anxiety scores revealed a predictive association between AChE–PON1 genotype and anxiety parameters [37]. Intriguingly, in healthy volunteers, the blood levels of soluble AChE-R monomers are inversely proportional to trait anxiety – that is, the tendency to become anxious that reflects a combination of demographic determinants (e.g. age, ethnic origin, gender and body–mass index) and experience-derived elements [37]. This further implies that the capacity to overproduce AChE-R in reaction to various stressors is an important determinant of ability to cope with anxious stimuli. In blood, AChE-R is rapidly inactivated [38], but it might be protected from both oxidative stress and inhibitory anti-cholinesterases by PON1 [39]. This explains why debilitating PON1 polymorphisms, which increase the risks of elevated state anxiety [37], also increase the risk of developing pesticide-induced Parkinsonism [40]. The freedom to overproduce AChE-R when needed thus mediates interactions of ACh signaling with both the glutamatergic and dopaminergic systems.
This implies that AD-related change(s) in general splicing-associated molecule(s) act upstream of either AChE or PSEN2.

PD is the most common neurodegenerative disorder that primarily affects motion control. PD involves degeneration of dopaminergic neurons in the substantia nigra, followed by loss of dopamine in the striatum. The rate-limiting enzyme in catecholamine synthesis, tyrosine hydroxylase (TH), marks clearly the main brain regions affected in PD (Figure 3a,iii). Because AChE activity is highest in the striatum (Figure 3a,iii), with substantial overlap between cholinergic and dopaminergic neurons, the cholinergic system is closely involved with PD pathology. AChE–PON1 interactions have been shown to increase the risk of anti-AChE-induced PD [40]. Moreover, exposure to AChE inhibitors, closed-head injury and psychological stress (all leading causes of PD) are associated with imbalanced AChE alternative splicing and AChE-R overexpression. That TgR mice, unlike TgS mice, seem to be neurologically protected suggests an adaptive role for AChE-R in aging and neurodegenerative diseases. High levels of soluble and diffusible AChE-R could potentially scavenge circulating and diffusing AChE inhibitors following exposure, thus acting as a protecting agent and alleviating toxicity symptoms, at least for a while. However, prolonged AChE-R excess could also lead to damage.

MG is an autoimmune disorder in which circulating autoantibodies against nicotinic acetylcholine receptors (nAChRs) lead to NMJ malfunction, characterized by progressive reductions in the amplitude of responses and by fluctuating weakness and fatigue of voluntary muscles. Treatment of MG currently involves cholinesterase inhibitors and often immune-directed treatment with either thymectomy or high-dose corticosteroids. AChE is thus directly related to both the pathophysiology and the treatment of MG [46]. Specifically, AChE-R, but not AChE-S, is overexpressed in the muscles of animals with experimental autoimmune MG. In these rats, antisense suppression of AChE-R resulted in: improved baseline compound muscle action potentials, measured by electromyography; improved stamina, measured by treadmill exercise; and improved clinical scores, determined by weight gain of treated animals [21]. In Cynomolgus (macaque) monkeys (Macaca fascicularis), such treatment further suppressed neuronal pro-inflammatory cytokines [47], extending to the CNS Tracey’s immunological concept of the suppression by ACh of inflammatory reactions [48].

In AD, PD and MG, AChE is directly involved in the pathophysiology of the disease, in the treatment of the disease, or both. Interestingly, splicing modifications always seem to have a chief role, leading to AChE-R accumulation. Targeted isoform-specific therapy could thus have profound implications for AChE-related neuropathologies.

**Stress-induced changes in neuronal alternative splicing**

Splicing aberrations have been reported in many diseases [49] and in aging [50], but the underlying molecular mechanism remains largely obscure. How does stress trigger alternative splicing of neuronal AChE and accumulation of AChE-R? To address this question, one should examine the combined consequences of stress on constitutive splicing (Box 2), alternative splicing and splicing factors.

Various stressful stimuli involve regulated, specific modulations in neuronal alternative splicing [51]. For example, perinatal manipulations and postnatal handling both selectively elevate levels of glucocorticoid receptor (GR) mRNA containing a hippocampus-specific exon that facilitates adaptation to modified stimuli [52]. Depending on neuronal depolarization, glucocorticoids also regulate splicing of the murine Slo gene (also known as Kcnc1), which encodes a brain K⁺ channel [53]. Splice-site selection hence serves as a physiological adaptation to a change in the external conditions, while also contributing to the corresponding pathophysiological events. The fine balance between the splicing factors, more than the precise concentration of each specific protein, is the key regulatory determinant. Therefore, differential availability of splicing-related proteins probably alters the pattern of alternative splicing. Alternative splicing might thus be viewed as a beneficial adaptation strategy that modifies a finely tuned regulation process; alternatively, or in addition, its activation might induce adverse consequences of the modified processing of pre-mRNA [50].

The specific splicing of the glucocorticoid receptor, SLO K⁺ channel and PSEN2 under stress supports the notion of adaptation, because alternative splicing in these instances facilitates beneficial stress responses, at least in the short term. Stress-induced changes in the alternative splicing of AChE pre-mRNA and the over-production of AChE-R are likewise beneficial in acute stress responses, because AChE-R downregulates stress-induced hyperarousal [8]. However, under prolonged stress
conditions, numerous physiological functions are adversely suppressed, with crucial consequences for the affected organism. Similarly, transgenic animal studies [23,27] and animal disease models [21,54] suggest that prolonged overproduction of AChE-R could take its toll.

In seeking splicing-related proteins that might be responsible, at least in part, for the mammalian post-stress effects, the general splicing factor SC35 was recently found to have expression patterns comparable to those of AChE-R in post-stress brain neurons (Figure 4). Moreover, SC35 shifted alternative splicing of AChE from AChE-S to AChE-R mRNA in vitro [55]. The splicing factor ASF/SF2 caused the opposite effect in cultured cells, driving splicing towards the AChE-S transcript (Figure 4). Recently, these two factors have been shown to influence alternative splicing of the glutamate receptor subunit 2 (GluR2), regulating the flip–flop isoform ratio of this protein [56]. An intriguing example of neuronal alternative splicing regulation comes from studies on neuro-oncological ventral antigen 1 (NOVA1), an RNA-binding protein that regulates alternative splicing of the GABA<sub>A</sub> receptor γ2 subunit through direct binding to UCAU-rich intrinsic splicing enhancers. Importantly, NOVA1 is a master regulator of synapse-related transcripts [57] and in cases where NOVA levels falter (e.g. in the autoimmune disease paraneoplastic opsoclonus myoclonus ataxia, where anti-NOVA antigens are produced), abnormal neurological motor activity is observed, demonstrating the downstream consequences of perturbation of essential RNA-processing proteins.

**Concluding remarks and future perspectives**

The variety of AChE alternative transcripts highlights AChE as a complex molecule with a tightly regulated pattern of expression. The uniqueness of each variant is accentuated by its expression timing (e.g. during health or disease, or during normal or stressful situations), its expression compartment (e.g. in neurites or on synaptic membranes) and its interaction partners (e.g. PRiMA, GPI and RACK1). These three factors add an extra dimension to the intricate control of variant AChE proteins.

The recurrent involvement of AChE in various pathophysiological states, including neuropathologies and stress-related syndromes, places specific AChE variants as the main target of drug development and therapy for such disorders. The most frequently used compounds in such therapies are cholinesterase inhibitors (e.g. carba-mates), which target the AChE active site and prevent the rapid degradation of ACh [58]. However, these drugs (or the chemically similar anti-cholinesterase insecticides [1]) occasionally have less desirable side effects [58], and their neurotoxicity – which most probably involves interactions of the cholinergic and glutamatergic systems [59] – pertains even at low doses [60]. Antisense-mediated degradation of AChE mRNA has been used successfully to prevent AChE-R overexpression and to alleviate symptoms in a closed-head injury model in mice [54], in a rat model of MG [21], and in mice experiencing enhanced stress-induced memory impairments and fear [28]. One of the immediate advantages of targeting the mRNA rather than the processed protein is that minute drug concentrations can be used, minimizing side effects. Variant specificity could also be used to modulate a specific protein partner rather than all AChE isoforms. With this in mind, it will be important to study the expression and roles of the recently identified 5′-spliced AChE transcripts and protein products [13] in AChE-related neuropathologies, and to identify their disease-specific variants. Molecular targeting of these novel proteins could have important implications for therapy. Small interfering RNAs (siRNAs), through the endogenous RNA interference (RNAi) machinery, can also be used to target specific AChE transcripts. Such RNAi-mediated AChE silencing has recently been used successfully to knockdown AChE expression in a human colon adenocarcinoma cell line [61], and effective systemic administration of siRNA has also been reported [62]. Characterization of the related transcripts of AChE, initial dissection of the molecular pathways involved and the emergence of new molecular tools might thus hold promise and hope for those seeking treatments for stress-related and AChE-related neuropathologies.

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[Figure 4. Proposed molecular mechanism for stress-induced alternative splicing of AChE. In normal situations (left), the balance between the different splicing factors, primarily ASF/SF2 (purple) and SC35 (blue), results in regulated splicing of AChE, yielding mostly AChE-S mRNA (purple arrow) and AChE-S protein (green). Repeated psychological stress in mice (right), results via an unknown mechanism in prolonged overexpression of SC35, which interacts with a specific exonic splicing enhancer, favoring production of AChE-R mRNA (blue arrow) and generating mostly AChE-R protein (red, bottom) [55].]
59 Popke, E.J. (2003) From anticholinesterase toxicity to Alzheimer’s disease: important interactions of cholinergic and NMDA receptor systems. Toxicol. Sci. 72, 185–187
61 Park, S.E. et al. (2004) Acetylcholinesterase plays a pivotal role in apoptosis formation. Cancer Res. 64, 2652–2655
66 Li, H.B. et al. (1996) NMDA- but not AMPA-receptor antagonists augment scopolamine-induced spatial cognitive deficit of rats in a radial maze task. Brain Res. 725, 268–271
70 Bell, M.I. et al. (2002) Functional and molecular characterization of metabotropic glutamate receptors expressed in rat striatal cholinergic interneurons. J. Neurochem. 81, 142–149
71 Bonsi, P. et al. (2005) Modulatory action of metabotropic glutamate receptor (mGluR) 5 on mGluR1 function in striatal cholinergic interneurons. Neuropharmacology 49(Suppl. 1), 104–113
74 Yost, H.J. and Lindquist, S. (1986) RNA splicing is interrupted by heat shock and is rescued by heat shock protein synthesis. Cell 45, 185–193

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