ACHE ANTISENSE OLIGONUCLEOTIDE AS AN ANTI-INFLAMMATORY AGENT

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ABSTRACT

Disclosed is a novel use for AChE antisense oligonucleotides as anti-inflammatory agents, wherein said oligonucleotides are preferably as denoted by SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:7. Methods of treatment of inflammatory conditions, as well as fever, and particularly inflammation-associated neuropathies such as Guillain-Barré Syndrome, are described.
Fig. 1A

Fig. 1B

Fig. 1C

Fig. 1D

Fig. 1E

Fig. 1F
Fig. 3A

Cent. can.
Dh
Iz
Vh

Fig. 3B

hEN101, µg/Kg

none
150 P.O.
500 P.O.
500 I.V.

AChE-R pos. ce.
% sq. gr.

Fig. 3C

Ce. Bo. Diam. µm

10-20
* 20-40
↓ >40
Fig. 8A

Fig. 8B

Fig. 8C
Fig. 9A

Fig. 9B

r = -0.63

Fig. 9C

r = 0.68

Plac.  Endot.
1 hour

Fig. 11A

endotoxin

mediated temporal lobe (impaired declarative memory)

AChE activity

ACh vesicular

ACh synaptic

neocortex (activated working memory)

3 hour

Fig. 11B

cytokines

medial temporal lobe (impaired declarative memory)

AChE activity

9 hour

Fig. 11C

mediated temporal lobe (impaired declarative memory)

AChE-R proteases
Fig. 12A

An. T. (°C)

T. p. anest. (min)

Aver. An. T. (°C)

T. p. anest. (min)

Fig. 12B
Fig. 14A

Fig. 14B

Fig. 14C
Fig. 16A

![Bar charts showing IL-1 and IL-6 levels over time.](image)

Fig. 16B

![Diagram illustrating relationship between IL-1 and depression.](image)

Fig. 16C

![Diagram showing correlations between IL-1 and various cognitive functions.](image)
Fig. 18
ACHE ANTISENSE OLGONUCLEOTIDE AS AN ANTI-INFLAMMATORY AGENT

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

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FIELD OF THE INVENTION

[0002] The present invention relates to the field of anti-inflammatory agents. More specifically, the present invention provides a novel use for an antisense oligonucleotide targeted to the coding domain of the acetylcholinesterase (ACHE) nucleotide sequence, as an anti-inflammatory agent.

BACKGROUND OF THE INVENTION

[0003] All publications mentioned throughout this application are fully incorporated herein by reference, including all references cited therein.

[0004] Inflammation plays a crucial role in defense against pathogen invaders as well as in healing and recovery processes following various types of injury. However, the magnitude and duration of inflammatory responses have to be tightly regulated, because excessive inflammatory reactions can be detrimental, leading to autoimmune diseases, neurodegeneration, sepsis, trauma and other pathological conditions. It has long been recognized that regulation of inflammatory reactions is mediated both by immune responses (particularly the secretion of anti-inflammatory cytokines) and by neuroendocrine factors, particularly the activation of the pituitary-adrenal axis and the secretion of glucocorticoids. Recently it became evident that neural mechanisms are also involved in limiting inflammatory responses. In particular, it was found that cholinergic neurons inhibit acute inflammation, providing a rapid, localized, and adaptive anti-inflammatory reflex system (Tracy, 2002).

In the periphery, acetylcholine (ACH) is mainly released by the efferent vagus nerve. It significantly attenuates the production of the pro-inflammatory cytokines TNFα, interleukin-1β (IL-1β), IL-6 and IL-18, but not the anti-inflammatory cytokine IL-10 [Tracey, K. J. (2002) Nature 420, 853-859]. Reciprocally, IL-1 causes AChE over-production both in PC12 cells and in the rat cortex [Li, Y. et al. (2000) J. Neurosci. 20, 149-155], suggesting a closed loop whereby ACh suppresses IL-1, abating the induction of AChE production.


[0006] Because spinal cord motoneurons respond to ACh, the presumed quality control process should exert regulatory effects upon cholinergic neurotransmission. As it needs to function rapidly, it likely involves short-lived molecules. Furthermore, in order to be broad-ranged, the proposed mechanism is likely to be induced under widely diverse stressors. The normally rare, stress-induced acetylcholinesterase variant AChE-R meets all of the requirements from an inducer of such response(s). AChE-R is overproduced under psychological, chemical and physical stresses [reviewed by Soreq, H. & Seidman, S. (2001) Nat. Rev. Neurosci. 2, 294-302]. A parallel stress response involves down-regulation of choline acetyltransferase (ChAT) [Kaufman, D. et al., (1998) Nature 393, 373-377] and the genomically linked vesicular acetylcholine transporter (VACHT) [Weihe, E. et al. (1996) Proc. Natl. Acad. Sci. USA, 93, 3547-3552], together limiting the production and vesicle packaging of acetylcholine while expediting its degradation. This yields down-regulation of the cholinergic hyperexcitation that is associated with many stresses. At a longer range, this stress response is associated with hyperresponsivity to both agonists and antagonists of cholinergic neurotransmission [Meszoros, E. et al. (2002) Science 295, 508-512] and abnormal locomotor activities that can be ablated under antisense destruction of AChE-R mRNA [Cohen, O. et al. (2002) Mol. Psychiatry 7, 874-885]. Finely-tuned control over AChE-R levels thus emerged as a key component of stress management by spinal cord motoneurons. AChE-R over-expression, which suppresses ACh levels, further lead to increased IL-1 production. Should this be the case, antisense suppression of AChE-R production [Brenner, T. et al. (2003) Faseb J. 17(2), 214-22] would increase ACh levels and reduce the levels of pro-inflammatory cytokines in CNS neurons.


Moreover, mice that overexpress both AChE-S and AChE-R present progressive dendritic and spine loss [Beeri R. et al. (1997) J. Neurochem. 69:2441-51], as well as altered anxiety responses [Erb C. et al. (2001) J. Neurochem. 77:638-46]. Furthermore, these mice display early-onset deficits in social recognition and exaggerated responsiveness to stressful insults. These can be briefly ameliorated by conventional anticholinesterase treatment or for longer periods by an antisense oligonucleotide capable of specifically inducing the destruction of AChE-R mRNA [Cohen (2002) id ibid.], suggesting that AChE-R is the primary cause. Thus, AChE-R production may lead to both positive and negative effects on cognition.

Stressful insults induce AChE-R production in the periphery as well (e.g., in the small intestines), and failure to induce this production, in response to aversive stimuli, results in hypersensitivity to relatively mild stressors [Shapira M. et al. (2000) Hum. Mol. Genet. 9:1273-1281]. This observation raised the possibility that peripheral AChE modulations may serve as a surrogate marker of endotoxin-induced changes in cognition as well. However, in plasma, proteolytic cleavage of AChE-R leads to the appearance in the serum of a short immunopositive C-terminal peptide which facilitates the hematopoietic stress responses [Grisaru, D. et al. (2001) Mol. Med. 7, 93-105]. Hence, the inventors investigated the effects of endotoxin administration on both AChE activity and AChE-R cleavage in healthy human volunteers and explored potential correlations between these parameters, the secretion of cytokines or cortisol, and changes with time in memory functions. In addition to declarative memory, which involves consciously accessible records of facts and events through concerted functioning of hippocampal and prefrontal structures [Kim and Diamond (2002) id ibid.], the inventors assessed the effects of endotoxin and its interactions with AChE cleavage on working memory, which involves temporary storage and manipulation of information necessary for cognitive functioning [Baddeley A. (1992) Science 255:556-9], and has been shown to involve prefrontal cholinergic mechanisms [Pare M. L. et al., (2000) Science 290:2315-9].

Peripheral neuropathies are caused by altered function and structure of peripheral motor, sensory or autonomic neurons. The main causes of neuropathy are entrapment (compression), diabetes and other systemic diseases, inherited disorders, inflammatory demyelinating, ischemic, metabolic, and paraneoplastic conditions, nutritional deficiency states, and toxin-induced derangement. One example of a peripheral neuropathy is the Guillain-Barré syndrome (GBS).

GBS is an acute inflammatory polyneuropathy. It is the most common cause of acute flaccid paralysis worldwide, with an annual incidence of 0.75 to 2 in 100,000 in the general population. GBS is suspected when a patient presents with progressive motor weakness and loss of deep tendon reflexes (areflexia). Other clinical features include sensory symptoms, cranial nerve involvement, autonomic dysfunction causing pulse and blood pressure changes, and respiratory failure, which is a major cause of morbidity and

[0013] In about two thirds of patients, symptoms are preceded by an antecedent infection, commonly an upper respiratory tract infection (40%) or gastroenteritis (20%) occurring 4 weeks prior to onset of disease [Group (1996) id ibid.; Rees, J. et al. (1995) N. Eng. J. Med. 333: 1374-1379]. According to this, GBS is thought to result from abnormal immune responses triggered by certain infections agents and directed towards the peripheral nerves [Seneviratne (2000) id ibid.] Interestingly, one recent report suggests that the clinical symptoms of drug poisoning by the AChE-inhibitor rivastigmine resemble those of GBS [Lai, M. W. et al. (2005) N. Engl. J. Med. 353:3].

[0014] The diagnosis of Guillain-Barré syndrome is based on clinical presentation, which is then supported by cerebrospinal fluid (CSF) analysis demonstrating elevated protein content and normal leukocyte cell count, indicating an inflammatory reaction. Electrophysiological studies then specify the clinico-pathological type according to evidence for damage of myelin, motor or sensory axons [Asbury and Comblath (1990) id ibid.].

[0015] Segmental demyelination, termed acute inflammatory demyelinating polyradiculoneuropathy (AIDP) is the most common type of Guillain-Barré syndrome, apparently mediated by lymphocytic and macrophage infiltration of the peripheral nerves [Griffin J. et al., (1995) Brain 118: (Pt. 3), 577-595; Honavar M., et al., (1991) Brain 114: ( Pt. 3), 1245-1269; Rees (1995) id ibid.] Demyelination is demonstrated by electrophysiological reduction of nerve conduction velocity, and subsequent remyelination is associated with recovery. In contrast to this, only minimal demyelination but prominent Wallerian-like degeneration with periaxonal macrophage infiltration are detected in axonal degeneration types of GBS, where motor axons exclusively or motor together with sensory axons, are damaged in acute motor axonal neuropathy (AMAN) [McKhan G. et al. (1993) Ann. Neurol. 33: 333-342] and acute motor sensory axonal neuropathy (AMSAN) [Griffin (1995) id ibid.], respectively. Accordingly, the electrophysiological features in these cases are reduced compound muscle action potential (CMAP) amplitude, and additionally, reduced sensory nerve action potentials in AMSAN, but preserved conduction velocity, indicating axonal dysfunction without demyelination. Both axonal neuropathies are characterized by rapidly progressive weakness, often with respiratory failure, but although AMAN patients usually exhibit good recovery [McKhan (1993) id ibid.], the recovery of AMSAN patients is generally slow and incomplete, considered to be the most severe form of GBS (Brown and Feasby (1984) Brain 107: (Pt. 1) 219-239).

[0016] Axonal degeneration types of GBS are often preceded by infection with Campylobacter jejuni (CJ), which is associated with a slow recovery, and severe residual disability [Rees (1995) id ibid.]. There are several serotypes of CJ, and the one most often isolated from GBS patients belongs to Penner serotype 19 (O:19) (Saiki, T. et al. (1997) J. Infect. Dis. 176: Suppl. 2, S129-134). The lipopolysaccharides (LPS) of CJ share ganglioside-like epitopes with ganglioside-surface molecules of peripheral nerves, and patients with GBS have anti-ganglioside antibodies, suggesting that “molecular mimicry” is the immunopathogenic mechanism of injury to the peripheral nerve fibers [Sheikh, K. et al. (1998) Annu. N.Y. Acad. Sci. 845: 307-321; Yuki N. et al. (1993) J. Exp. Med. 178: 1771-1775]. Nevertheless, although CJ-O:19 serotype is significantly associated with elevated anti-ganglioside antibody titers in the sera of the patients, no significant correlation was found between the presence of these antibodies and the clinical pattern of GBS [Nishimura M. et al. (1997) J. Neurol. Sci. 153: 91-99]. This therefore indicates that additional factors may determine the axonal damage or disfunction following the apparently antibody-mediated nerve-surface injury. In agreement with this, the currently accepted treatments of GBS is intravenous immunoglobulin administration or plasma exchange (plasmapheresis), which act through suppression or removal of auto-antibodies, both which have been found to be equally beneficial [Seneviratne (2000) id ibid.]. Nevertheless, several authors reported a rapid resolution of nerve conduction blocks following plasmapheresis, which could not be explained by remyelination or axonal regeneration [Kuwabara S. et al. (1999) Muscle Nerve 22: 840-845; Suzuki and Choi, (1991) Acta. Neuropatholog. (Berl) 82: 93-101]. This suggests a possible role for a humoral factor in the pathogenesis of the disease, causing physiological conduction abnormalities that may facilitate the destructive process.


[0018] The role of cholinergic mechanisms in learning and memory, the involvement of AChE-R in stress responses, the
suppression by ACh of pro-inflammatory cytokine production, the effects of endotoxin on memory functions, and the potential involvement of AChE-R in nerve conduction block, suggested involvement of AChE-R in inflammatory associated processes which could thus potentially be suppressed by an inhibitor of AChE-R expression.

[0019] The prospect of therapeutic agents of exquisite specificity and action at very low concentration has stimulated the development of antisense oligonucleotides (AS-ON) targeted against a variety of mRNAs. Major problems remain access to the RNA processing machinery of the cell, potential differences between specific cell types and the mode of chemical protection employed. When the cell of interest is within the CNS, the problem of access is compounded by the presence of the blood-brain barrier [Mavitian, B. et al. (1998) Nat. Med. 4, 467-471]. Nevertheless, some attempts have been successful even in primates [Kasuya, E. et al., (1998) Regul. Pept. 75-76, 319-325; Mizuno, M. et al. (2000) Endocrinology 141, 1772-1779]. The inventors have previously demonstrated antisense suppression of the stress-induced AChE-R mRNA, enabling retrieval of normal cellular and physiological functions following stress-induced changes in cultured rat and human cells [Galyam, N. et al. (2001) Antisense Nucleic Acid Drug Dev. 11, 51-57; Grisaru, D. et al. (2001) id ibid.] and in live mice [Cohen et al., (2002) id ibid.; Shohami, E. et al., (2000) J. Mol. Med. 78, 228-236] and rats [Brenner, T. et al. (2003) id ibid.]. While the tested consequences in all of these studies were limited to direct measurement of the target protein and mRNA, the working hypothesis predicted additional, anti-inflammatory effects for antisense retrieval of cholinergic balance. Here, the inventors report the outcome of experiments aimed at addressing the stress-induced overproduction and selective AS-ON retrieval of normal AChE-R levels under injection stress in cynomolgus monkeys. The findings demonstrate differential susceptibility of specific neuron types to AS-ON responses, as well as concomitant suppression of IFN-IFg and IFN-IFg following the retrieval of cholinergic balance in spinal cord neurons. The present inventors have previously found that antisense oligonucleotides against the common coding region of AChE are useful for suppressing AChE-R production [see Wo 98/26062]. In particular, the inventors have shown the use of an antisense oligonucleotide against the AChE sequence for the treatment of myasthenia gravis [Wo 03/002739].

[0020] Based on the inventors’ herein described results, the present invention provides a novel use for an antisense oligonucleotide directed against the AChE mRNA sequence, as a new anti-inflammatory agent, and particularly for the treatment of subjects afflicted with inflammation associated neuropathies such as the Guillain-Barré Syndrome.

[0021] Other purposes and advantages of the invention will become apparent as the description proceeds.

SUMMARY OF THE INVENTION

[0022] In a first aspect, the present invention refers to a method of treatment of conditions triggering an inflammatory response in a mammalian subject in need, comprising administering a therapeutic effective amount of an inhibitor of AChE expression, or a pharmaceutical composition comprising the same.

[0023] In particular, said conditions are selected from any one of stress, bacterial infection, drugs, irradiation, exposure to AChE inhibitors, stroke, auto-immune diseases, multiple chemical sensitivity and any cumulative age-dependent damages.

[0024] In another aspect, the present invention provides a method for the treatment and/or prevention of inflammation in the joints, central nervous system, gastrointestinal tract, endocardium, perocardium, lung, eyes, skin and urogenital system in a mammalian subject in need, comprising administering a therapeutic effective amount of an inhibitor of AChE expression, or a pharmaceutical composition comprising the same.

[0025] In a further aspect, the present invention provides a method for suppressing the release of pro-inflammatory cytokines, comprising administering a therapeutic effective amount of an inhibitor of AChE expression, or a pharmaceutical composition comprising the same, to a subject in need.

[0026] Preferably, said pro-inflammatory cytokine is selected from the group consisting of IL-1β, TNFα, IL-6, IL-8, IL-12 and IL-18, and the release of said cytokine is triggered by one of stress, bacterial infection, drugs, irradiation, exposure to AChE inhibitors, stroke, auto-immune diseases, multiple chemical sensitivity, and any cumulative age-dependent damages.

[0027] In an even further aspect, the present invention provides a method for treating fever, comprising administering a therapeutic effective amount of an inhibitor of AChE expression, or a pharmaceutical composition comprising the same, to a subject in need.

[0028] Further, the present invention also provides a method for the treatment of inflammation-associated neuropathies, comprising administering a therapeutic effective amount of an inhibitor of AChE expression, or a pharmaceutical composition comprising the same, to a subject in need. One particular example of an inflammation-associated neuropathy is the Guillain-Barré Syndrome.

[0029] In one embodiment of the methods of the invention, said inhibitor of AChE expression is one of an AChE-specific ribozyme, an RNA sequence used for RNA interference of the AChE gene, and an antisense oligonucleotide directed against AChE.

[0030] In one preferred embodiment of the methods of the invention, said inhibitor of AChE expression is a nuclease resistant antisense nucleotide directed against AChE, or functional analogs, derivatives or fragments thereof.

[0031] In another preferred embodiment of the methods of the invention, said inhibitor of AChE expression is an antisense oligonucleotide directed against AChE, having the sequence as denoted by any one of SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:7, as follows:

5'-CTGCCAGTCTCTGTCGACC-3' (SEQ ID NO:1)

5'-CTGCAAATTTTCTTGCGACC-3' (SEQ ID NO:2)

and

5'-CTGCCAATTTTCTTCGACA+C+C+3', (SEQ ID NO:7)

wherein the three 3' terminal residues are modified with 2-O-methyl groups (•), or functional analogs, derivatives or fragments thereof.
[0032] In a further preferred embodiment of the methods of the invention, said mammalian subject is a human, and said inhibitor of AChE expression is an antisense oligonucleotide directed against AChE, as denoted by the sequence selected from SEQ ID NO:1 and SEQ ID NO:7, or functional analogs, derivatives or fragments thereof.

[0033] In another embodiment of the method of the invention, said antisense oligonucleotide or composition comprising the same is for daily use by a subject in need, and said therapeutic effective amount is a dosage of active ingredient between about 0.001 μg/g and about 50 μg/g. Preferably, said dosage of active ingredient is between about 0.01 and about 5.0 μg/g. More preferably, said dosage of active ingredient is between about 0.15 and about 0.50 μg/g.

BRIEF DESCRIPTION OF THE FIGURES

[0034] FIG. 1A-1F: Reduced VACHT accumulation in cholinergic terminals and partition cells of treated monkeys.

[0035] FIG. 1A: Confocal microscopy projections of spinal cord motoneurons (cell diameter=40 μm), immuno-labeled (white) with anti-VACHT antibody. The total volume and average number per cell of labeled terminals were measured using Image-Pro Plus software, and the result of each treatment (1, 2, 3, and 4) plotted in the graphs shown in FIGS. 1B and 1C.

[0036] FIG. 1B: Average value of volume and average number per cell of labeled terminals, including all motoneurons detected in a section.

[0037] FIG. 1C: Population distribution of volume and average number per cell of labeled terminals, including all motoneurons detected in a section.

[0038] FIG. 1D: Average values of FIGS. 1B, 1C analyses (± Standard Evaluation of the Mean, SEM). Significant reductions are marked by asterisks (p<0.01, Student’s t test).

[0039] FIG. 1E: Immunolabeling with anti-ChAT antibody in partition cells from naïve spinal cord, localized in close proximity to the central canal (arrows). Hematoxylin was used for background staining.

[0040] FIG. 1F: Higher magnification of ChAT positive partition cells in naïve monkeys (1) or following oral (p.o.) administration of 150 μg/kg/day (2) or 500 μg/kg/day (3) and i.v. administration of 500 μg/kg/day hEN101 (4). Note dose-independent handling—induced reductions in both terminal volume and density.

[0041] Abbreviations: n., naïve; Tern., terminal; vol., volume; Part. Ce., Partition cell; Cent. Can., Central canal.

[0042] FIG. 2A-2J: Selective AChE-R mRNA suppression by hEN101 in monkey spinal cord neurons.

[0043] FIG. 2A: Scheme of the human AChE gene coding exons and two of its alternative transcripts, the synaptic AChE-S(S) and the stress-associated AChE-R(R) mRNA. The S transcript includes exons 2, 3, 4 and 6, whereas the R transcript contains exons 2, 3, 4, 5 and pseudointron 4'. These distinctions served to prepare transcript-specific probes, indicated by an asterisk.

[0044] FIG. 2B: Sampling site on the dissected monkey lumbar spinal cord is indicated by an arrow.

[0045] FIG. 2C-2J: Tissue sections from lumbar spinal cords were prepared following 7-day treatment with the noted doses of hEN101 by p.o. or i.v. administration. Shown is in situ hybridization used to compare neuronal labeling pattern with the noted probes. Nuclei were visualized by DAPI staining (white). There was no difference between tested sections in total cell numbers and/or general histology. Note that AChE-S mRNA labeling displayed significant changes following treatment only in neuronal process sections (2F, 2H and 2J as compared to 2D), whereas neuronal AChE-R mRNA labeling was notably reduced in cell bodies.

[0046] FIG. 2C: No treatment, staining specific for AChE-R mRNA.

[0047] FIG. 2D: No treatment, staining specific for AChE-S mRNA.

[0048] FIG. 2E: Treatment with 150 μg/kg/day of EN101, p.o., staining specific for AChE-R mRNA.

[0049] FIG. 2F: Treatment with 150 μg/kg/day of EN101, p.o., staining specific for AChE-S mRNA.

[0050] FIG. 2G: Treatment with 500 μg/kg/day of EN101, p.o., staining specific for AChE-R mRNA.

[0051] FIG. 2H: Treatment with 500 μg/kg/day of EN101, p.o., staining specific for AChE-S mRNA.

[0052] FIG. 2I: Treatment with 500 μg/kg/day of EN101, i.v., staining specific for AChE-R mRNA.

[0053] FIG. 2J: Treatment with 500 μg/kg/day of EN101, i.v., staining specific for AChE-S mRNA.

[0054] FIG. 3A-3C: Cell size-dependent efficacy of neuronal AChE-R mRNA suppression.

[0055] FIG. 3A: Scheme of the lumbar spinal cord and its three compartments: the ventral and dorsal horns separated by the intermediate zone and the central canal.

[0056] FIG. 3B: Histological staining (Hematoxylin and eosin) of a representative field in the intermediate zone of the lumbar spinal cord. Three cells are marked as according to their perikaryon diameters: 10-20 μm (arrow, the majority of those cells is located in the dorsal horn), 20-40 μm (asterisk) and =40 μm (arrow).

[0057] FIG. 3C: Shown as fractions of AChE-R positive neurons from the three size groups under the different treatment regimens. Insets: representative neurons from the different size groups, taken from the p.o. 150 μg/kg/day regimen. Columns show average AChE-R positive cells in each size group ±SEM representing repeated analyses of the entire lumbar spinal cord gray matter in multiple sections. Stars note significant differences (p<0.05, Wilcoxon test).

[0058] Abbreviations: Cent. Can., central canal; D. h., dorsal horn; I. z., Intermediate zone; V. h., ventral horn; pos. ce., positive cells; si. gr., size group; Ce. Bo. Diam., cell body diameter.

[0059] FIG. 4A-4C: Suppression of stress-induced pro-inflammatory cytokines under antisense intervention with AChE-R expression.

[0060] FIG. 4A: Shown are fractions of IL-1β positive spinal cord neurons of medium and large sizes under the different treatment regimens (columns ±SEM representing repeated analyses of the ventral horn and intermediate zone
of lumbar spinal cord gray matter in multiple sections). Insets: representative medium and large size positive neurons, taken from the p.o. 500 µg/kg/day regimen. *: p≤0.05, **: p<0.007.

[0061] FIG. 4B: Graph showing the correlation between the average fractions of AChE-R and IL-1β positive medium-sized cells (20-40 µm) in the different hEN101 treatments. Large cells (>40 µm) did not display such correlation (R²=0.1778).

[0062] FIG. 4C: Fractions of IL-6 positive spinal cord neurons were evaluated essentially as under 4A. Note decreases in both IL-1β and IL-6 in spinal cord neurons of monkeys treated with 500 µg/kg/day EN101.

[0063] Abbreviations: pos. ce., positive cells.

[0064] FIG. 5A-5D: Changes over time in the human plasma levels of AChE activity and in AChE-R cleavage.

[0065] FIG. 5A: Hydrolytic activities. Shown are plasma AChE activities (mean±SEM) for ten volunteers injected twice, with endotoxin or saline (placebo) at the noted intervals after injection. Pre-injection (baseline) AChE level was considered as 100% for each individual. Asterisks denote statistical difference (p<0.05).

[0066] FIG. 5B: Immunoblot. Shown are consecutive results for one individual. Plasma samples underwent electrophoresis by SDS-PAGE, and the blot immunoreacted with anti-AChE-R antibodies. Note the 6.5 kDa AChE-R cleavage product. Left lanes indicate the response to a placebo injection; right lanes demonstrate elevated AChE-R cleavage in response to endotoxin.

[0067] FIG. 5C: Densitometric intensities. Shown are average values (mean±SEM) of the rapidly migrating AChE-R cleavage product in plasma of the endotoxin and placebo treated individuals as % of baseline (described in A).

[0068] Note: Elevated AChE-R cleavage in endotoxin-treated subjects co-appeared with decreased AChE activity.

[0069] FIG. 5D: Association analysis. Highly significant negative association (correlation coefficient, r=−0.65) emerged between the increases in AChE-R cleavage and the decreases in AChE activity under endotoxin during the last testing period (t=9 hr). Each dot represents a single individual.


[0071] FIG. 6: Mass spectroscopy of gel-eluted band.

[0072] Shown is the outcome of electron spray mass spectrometry analysis of the gel-eluted rapidly migrating band that immunoreacted with anti-AChE-R antibodies. Note that the main peptide displayed a molecular mass of 3613-3615. Calculation of predicted masses positioned the presumed proteolytic cleavage site 36 residues from the C-terminus of AChE-R, between asparagine and arginine residues in the sequence presented, with the presumed cleavage site arrowed and the diversion site starred.

[0073] Abbreviations: Rel. abund., relative abundance.

[0074] FIG. 7A-7C: AChE-R is expressed in human vascular endothelial cells from various tissues.

[0075] FIG. 7A: AChE-R mRNA. Shown are the results of in situ hybridization using a 5'-biotinylated cRNA probe selective for the AChE-R mRNA variant on sections of human vascular endothelial cells affected by an inflammatory process (skin hypersensitivity vasculitis; labeling is seen as pink color, red arrow).

[0076] FIG. 7B: AChE-R protein. Shown is an immunohistochemical section of human kidney vascular endothelial cells from a patient with vasculitis, labeled with antibodies targeted at the AChE-R C-terminal peptide (red arrow).

[0077] FIG. 7C: Image analysis. Shown are average AChE-R mRNA and AChE-R protein labeling intensities (black and white columns, respectively), in kidney, skin and muscle vascular endothelial cells (mean values±SEM) as the percentage of red pixels, falling within a defined intensity range.

[0078] Abbreviations: prot., protein; int., intensity; k. rej., kidney rejection; k. vas., kidney vasculitis; nonspec., non-specific; n. end., normal endothelium; m., muscle; hyp. vac., hypersensitivity vasculitis.

[0079] FIG. 8A-8C: Bidirectional associations between AChE-R cleavage and the changes in cortisol and cytokines.

[0080] Shown are average ±SEM changes with time (left) in the plasma levels of cortisol, TNF-α and IL-6 of the 10 patients treated with endotoxin or placebo, and the associations (right) at the noted time points between these changes and the changes in AChE-R cleavage (measured by densitometric quantification of the C-terminus AChE-R cleavage product).

[0081] FIG. 8A: cortisol.

[0082] FIG. 8B: TNF-α.

[0083] FIG. 8C: IL-6.

[0084] Abbreviations: r, correlation coefficient; t, time after injection; Plac., placebo; end., endotoxin; H. p. inj., hours post-injection; cleav. prod., cleavage product.

[0085] FIG. 9: Endotoxin impairs declarative memory. Shown are average ±SEM values for the performance in the immediate story recall test of the endotoxin and placebo treated individuals at the noted time following treatment as well as the associations of the changes in these values at 9 hr post-injection with the changes in AChE-R cleavage (b) and AChE activity (c).

[0086] Abbreviations: I.s.r., immediate story recall; plae., placebo; endot., endotoxin; H.p.inj., hours post-injection; cleav. prod., cleavage product; act., activity.

[0087] FIG. 10: Endotoxin-induced improvement in working memory.

[0088] Shown are the performance values (average ±SEM) in the span background test for the endotoxin and placebo treated individuals (a) and the association of the changes in this performance at 3 hr post-injection with the changes in AChE-R cleavage (b).

[0089] Abbreviations: r, correlation coefficient; t, time after injection; S.b., span backward; plae., placebo; endot., endotoxin; H.p.inj., hours post-injection; cleav. prod., cleavage product; act., activity.
[0090] FIG. 11A-11C: Scheme—Endotoxin induces inter-related cytokine-cholinergic effects on memory.

[0091] Shown are the cellular and biochemical events that were explored in this study and which explain the changes in memory processes and the dynamic modifications in these changes during the post-treatment observation period. The thickness of arrows reflects the relative intensity of the relevant processes.

[0092] FIG. 11A: At 1 hr post-treatment: Endotoxin induces the release of cytokines, cortisol and proteases. Cytokines elevate associated with impaired declarative memory, which is a medial temporal lobe-associated phenomenon. Cortisol induces AChE-R production, which elevates the immunopositive AChE-R amounts in plasma. Vesicular ACh is released into the synaptic cleft, where it affects neuronal electrophysiology and may improve working memory, which is a neocortex-associated property. In the periphery, ACh begins to suppress cytokines production in macrophages (circumolar arrow).

[0093] FIG. 11B: At 3 hr post-treatment: Proteases release a C-terminal fragment of 36 amino acids in length from AChE-R and initiate further destruction, followed by decreases in AChE activity. Endotoxin is already gone, and ACh effectively suppresses cytokines production. Increased ACh levels (reflecting enhanced secretion and the decrease in AChE’s hydrolytic activity) are probably associated with activated working memory, whereas the elevation in AChE-R cleavage product is associated with a lower working memory improvement.

[0094] FIG. 11C: At 9 hr Post-treatment: Cortisol is gone as well. However, the persistent, although slow decrease in AChE activity is associated both with the impaired declarative memory and, probably through ACh increases, with the activated working memory. The steady increase in AChE-R cleavage product is now associated both with a greater impairment in declarative memory and with lower improvement in working memory.

[0095] Abbreviations: inc. lev., increased level; dec. lev., decreased level; cleiu. Prod., cleavage product.

[0096] FIG. 12A-12B: Transgenic mice display higher body temperature than wild-type mice.

[0097] FIG. 12A: Graph showing the temperature of each mouse over time, squares represent transgenic mice, circles, control.

[0098] FIG. 12B: Graph showing the average temperature of each group (transgenic or control) over time, diamonds represent transgenic mice, squares, control.


[0100] FIG. 13A-13C: Effects of Tacrine on LPS-induced IL-1 secretion in the hippocampus and IL-1 and TNF-α secretion in the serum.

[0101] FIG. 13A: Graph showing the levels of IL-1β in the hippocampus.

[0102] FIG. 13B: Graph showing the levels of IL-1β in the serum.

[0103] FIG. 13C: Graph showing the levels of TNF-α in the serum.

[0104] Abbreviations: prot., protein; ser., serum; sal., saline.

[0105] FIG. 14A-14C: Effects of Rivastigmine on LPS-induced IL-1 secretion in the hippocampus and IL-1 and TNF-α secretion in the serum.

[0106] FIG. 14A: Graph showing the levels of IL-1β in the hippocampus.

[0107] FIG. 14B: Graph showing the levels of IL-1β in the serum.

[0108] FIG. 14C: Graph showing the levels of TNF-α in the serum.

[0109] Abbreviations: prot., protein; ser., serum; sal., saline.


[0111] FIG. 15A: Graph showing the effect of surgery stress on anxiety.

[0112] FIG. 15B: Graph showing the effect of surgery stress on depression.

[0113] FIG. 15C: Graph showing the effect of surgery stress on fatigue.

[0114] FIG. 15D: Graph showing the effect of surgery stress on pain.

[0115] FIG. 15E: Graph showing the effect of surgery stress on word list recall.

[0116] FIG. 15F: Graph showing the effect of surgery stress on word list recognition.

[0117] FIG. 15G: Graph showing the effect of surgery stress on story recall.

[0118] FIG. 15H: Graph showing the effect of surgery stress on figure recall.

[0119] Abbreviations: Cont., control; str., stress; T., time; Aux., anxiety; Dep., depression; Fat., fatigue; P., pain; W.L.R., word list recall; W. L. Recog., word list recognition; S. R., story recall; Fig. R., figure recall.

[0120] FIG. 16A-16C: Effect of surgery stress on cytokine levels.

[0121] FIG. 16A: Graph showing the effect of surgery stress on IL-1 and IL-6 levels.

[0122] FIG. 16B: Correlation between IL-1 and depression.

[0123] FIG. 16C: Correlation between cytokines and cognitive parameters.

[0124] FIG. 17A-17C: Reduction of AChE gene expression upon EN301 treatment.

[0125] FIG. 17A: Analysis of RT-PCR reaction (AChE exon 2 product after 31 PCR cycles). From left to right: lane 1, marker; lanes 2-8, samples from EN301-treated mice; lanes 9-14, samples from PBS-treated mice.
FIG. 17B: Histogram representing quantitative analysis of the results obtained in the PCR reaction using primers targeting the common sequence in exon 2 of murine AChE cDNA.

FIG. 17C: Histogram representing quantitative analysis of the results obtained in the PCR reaction using primers targeting the sequence in exon 6 unique to the AChE-S variant.

Abbreviations: c.d., common domain; Arb. U., arbitrary units; sal., saline.

FIG. 18A-18C: Schematic representation of injections and conduction tracings in the GBS model.

FIG. 18A: Systemic exposure was provided by intra-peritoneal (i.p.) injection (systemic injection). Intraneural (i.n.) injections were to the sciatic nerve at the mid thigh level.

FIG. 18B: Compound muscle action potential is recorded from the intraneural foot muscles following proximal stimulation of the sciatic nerve at the sciatic notch and distal stimulation of the peroneal and posterior tibial nerves at the ankle.

FIG. 18C: Proximal to distal amplitudes ratio (PDR) of less than 0.5 indicates conduction block.

Abbreviations: i.n., intraneural; i.p., intraperitoneal; LPS, lipopolysaccharide; EN101 or AS, antisense oligonucleotide; SM, splenocyte medium; BMM, bone marrow macrophage; ARP, AChE-readthrough peptide; ASP, AChE-synaptic peptide; n., none; sal., saline; inj., injection; PID2, second post-injection day; INj., intra-neural injection; LPS-R, lipopolysaccharide-reactive.

FIG. 19A-19D: Histograms representing average proximal to distal amplitude ratio (PDR) in selected experiments.

Measurements were obtained one day following intra-neural injection or on the second post-injection day, where designated.

Abbreviations: i.n., intraneural; i.p., intraperitoneal; LPS, lipopolysaccharide; EN101 or AS, antisense oligonucleotide; SM, splenocyte medium; BMM, bone marrow macrophage; ARP, AChE-readthrough peptide; ASP, AChE-synaptic peptide; n., none; sal., saline; inj., injection; PID2, second post-injection day; INj., intra-neural injection; LPS-R, lipopolysaccharide-reactive.

FIG. 20: Immunoblot signal for PKCB II.

Expression of PKCβII is increased following intraneural injection (i.n.) of LPS-reactive splenocyte medium in two nerves (RM, lanes 3, 4) compared to two nerves injected with non-reacted splenocyte medium (NM, lanes 1, 2). This increase is attenuated in two nerves by concomitant i.n. injection of antisense EN101 (RM+EN101, lanes 5, 6).

DETAILED DESCRIPTION OF THE INVENTION

For the purposes of clarity, the following abbreviations and terms are defined herein:

AChE: acetylcholinesterase

AChE-R: acetylcholinesterase, "readthrough" variant or isoform, its mRNA includes pseudo-intron

AS-ON: antisense oligonucleotide

CMAP: compound muscle action potential

CNS: central nervous system

EN101: may also be referred as AS3, antisense oligonucleotide targeted against human, rat or mouse (hEN101, rEN101 or mEN101, respectively) AChE mRNA

EN301: may also be referred as mEN101, antisense oligonucleotide targeted against mouse AChE mRNA

GBS: Guillain-Barré Syndrome

i.n.: intraneural

i.p.: intraperitoneal

i.v.: intravenous

o.g.: oral gavage

p.o.: per os

PDR: proximal to distal amplitude ratio

Antisense oligonucleotide: A nucleotide comprising essentially a reverse complementary sequence to a sequence of AChE mRNA. The nucleotide is preferably an oligodeoxynucleotide, but also ribonucleotides or nucleotide analogues, or mixtures thereof, are contemplated by the invention. The antisense oligonucleotide may be modified in order to enhance the nuclease resistance thereof, to improve its membrane crossing capability, or both. The antisense oligonucleotide may be linear, or may comprise a secondary structure. It may also comprise enzymatic activity, such as ribozyme activity.

To reveal if cholinergic allostatics and CNS inflammatory processes are inter-related, the inventors studied spinal cord neurons from Cynomolgus monkeys following one week daily treatment with hEN101 (SEQ ID NO:1), a 2'-oxymethylated antisense oligonucleotide inducing AChE-R mRNA destruction. hEN101 prevented the stress-induced increases in plasma AChE activities and selectively suppressed neuronal AChE-R mRNA and interleukins-1β and -6 levels in a dose- and cell size-dependent manner. In contrast, VACHT and ChAT levels were reduced dose-independently in all of the handling-stressed monkeys, demonstrating distinct regulation for the corresponding genes. These findings allude to a causal association between cholinergic allostatics and inflammatory responses in the primate CNS and suggest antisense intervention with AChE-R accumulation for the management of both these impairments. Furthermore, EN101 intervention in a GBS model prevented the appearance of nerve conduction block following both in vivo and in vitro exposure to Cj-LPS, and had a similar effect by exposure to E. Coli LPS.

Thus, the present invention refers to the use of an inhibitor of AChE expression, as an anti-inflammatory agent. Mainly, the present invention provides methods of treatment and/or prevention of conditions selected from the group consisting of: conditions triggering an inflammatory response; inflammation, release of pro-inflammatory cytokines, fever, and inflammation-associated neuropathies, par-
ticularly GBS, said method comprising administering a therapeutically effective amount of an inhibitor of AChE expression, or a pharmaceutical composition comprising the same, to a subject in need.

[0158] As herein defined, an inhibitor of AChE expression is any agent which is capable of blocking or hindering the expression of the AChE gene, particularly by interacting with its mRNA. Thus, said inhibitor may be an AChE-specific ribozyme, a double-stranded nucleotide sequence used for RNA interference of the AChE gene, or an antisense oligonucleotide directed against AChE. Antisense nucleotides are preferably nucleoside resistant.

[0159] Preferably, said inhibitor of AChE expression selectively inhibits the AChE-R mRNA, consequently selectively inhibiting the expression of the AChE-R isomorph. In this regard, any agent capable of inhibiting the soluble AChE-R isomorph may also be an anti-inflammatory agent. Therefore, a putative molecule that could block AChE-R expression and/or function would be an anti-inflammatory agent.

[0160] As shown in Example 1, BuChE levels in the plasma of treated monkeys were not significantly altered, supporting the notion of a selective antisense effect over AChE alone. Both plasma AChE activity and neuronal AChE mRNA labeling increased in monkeys treated with 150 μg/kg hEN101, potentially reflecting increased production at the tested daily time (Table 1 and data not shown). Alternatively, or in addition, the mild stress associated with the insertion of cannula for p.o. administration of hEN101 could be the cause. Plasma AChE increases in the absence of hEN101 would likely be even higher, as is indicated from the suppression of plasma AChE activity in monkeys treated similarly with the higher dose of 500 μg/kg hEN101. An apparent 3 hr delay was observed in the drug-induced decreases of plasma AChE under this low hEN101 dose, possibly reflecting prevention by antisense agents of the synthesis of their target protein(s). This further indicates a short half life for primate AChE-R mRNA in vivo, compatible with previous findings by the inventors and others [Brenner et al. (2003) id ibid.; Chan, R. Y. et al. (1998) J. Biol. Chem. 273, 9727-9733].

[0161] The fraction of AChE-R mRNA positive neurons, the intensity of AChE-R mRNA labeling and the fraction of cells with AChE-R mRNA labeled processes were all reduced under antisense treatment (FIGS. 2A-2J and 3A-3C). Neuronal susceptibility of AChE-R overproduction to antisense suppression appeared inversely proportional to cell body size, possibly reflecting distinct membrane and/or metabolic properties, different cell volumes or a combined contribution of these properties. In addition, antisense-independent reductions in VACHT and ChAT likely indicated a slowdown of vesicle recycling [Soreq, H. et al. (1990) Proc Natl Acad Sci U.S.A. 87: 9688-9692], potentially modulating the pace of cholinergic neurotransmission. Under naive conditions, AChE-S mRNA appeared in processes of many more spinal cord neurons than AChE-R mRNA, creating a pattern reminiscent of VACHT labeling in the rat spinal cord ventral horn [Weltje et al. (1996) id ibid.]. Expectedly, hEN101 treatment was highly efficient with neuronal AChE-R mRNA and much less effective with AChE-S mRNA. However, the reduced intensity of neuronal AChE-S mRNA labeling likely reflected limited reduction in neuronal AChE-S mRNA levels as well. Under hEN101 treatment, AChE-S mRNA in processes was reduced, suggesting common tendency for reduced dendrite translocation of the rodent and primate AChE-S mRNA transcript under stress [Mesher et al. (2002) id ibid.]. This difference further strengthened the notion that the naive monkey was indeed under no stress, an important fact in a study with strictly limited number of animals. The reduced AChE-S mRNA in neuronal processes of the treated monkeys might be treatment- and/or drug-induced. Following 7 days treatment, a shift from the primary AChE-S mRNA transcript to the stress-induced antisense-suppressible AChE-R mRNA may be visualized in the neuronal processes (FIG. 2A-2J).

[0162] Preferably, said inhibitor of AChE expression is an antisense oligonucleotide directed against AChE, having any one of the following sequences:

\[
5' \text{GTCAGCTCTCCTGAGCC} 3'; \quad (\text{SEQ ID NO11})
\]

\[
5' \text{GTCAGCTCTCCTGACCA+} 3', \quad (\text{SEQ ID NO17})
\]

wherein the three 3' terminal residues are modified with 2-O-methyl groups (*).

[0163] The antisense oligonucleotides denoted by SEQ ID NO:1 or SEQ ID NO:7 are also referred to herein as EN101, or hEN101. hEN101 is also commercially known as Monarsen™.

[0164] The antisense oligonucleotides directed against AChE have been described in the past by the present inventors [WO03/002739], and were shown to have a potent effect in the treatment of the neuromuscular pathology myasthenia gravis [applicant’s co-pending U.S. 2003-0216344]. In the inventors’ herein described results, as shown in Example 5 and FIG. 4, the antisense oligonucleotide directed against AChE was able to reduce the release of IL-1β, which is a pro-inflammatory cytokine.

[0165] As shown in Example 1, AChE-R mRNA levels in motoneurons were minimally affected. However, elimination of AChE-R production in spinal cord smaller neurons potentially increased ACh signaling within the treated tissue, in spite of the stress-induced reduction in VACHT and ChAT [Kaufler et al. (1998) id ibid.]. This attributes to AChE-R the primary role of regulating ACh levels in the CNS. Findings of others show large variability in the electrophysiological activity patterns of spinal cord interneurons [Perlmuter (1996) id ibid.] as well as pre-movement instructed activity in them [Prutt and Fetz (1999) id ibid.]. The inventors observed the largest variability in AChE-R levels within small cells, probably interneurons, suggesting that these modulations may contribute towards the wide electrophysiological variability between these neurons. Under normal conditions, AChE-R expression in small cholinergic neurons, localized to the dorsal horn of the spinal cord, may thus contribute to the control of motoneuron activities (e.g. motor reflexes). C-terminal structures, which affect the cholinergic input to motoneurons, were considered to originate in proximity to the motoneurons themselves [Hellstrom (1999) id ibid.]. This study attributes this origin to AChE mRNA positive interneurons and small cholinergic neurons located in the ventral horn and intermediate zone of the lumbar spinal cord. The numbers of VACHT-labeled C-terminals
surrounding motoneuron cell bodies decreased in all of the handled animals. This observation attributes this decrease to the handling stress, compatible with the stress-induced decreases in ChAT and VACHT mRNA in hippocampal neurons [Kauf et al. (1998) ibid.].

Additional antisense oligonucleotides directed against AChE have also been described, and potentially have the same anti-inflammatory effect as hEN101, as demonstrated in Example 16 for mEN101. These are antisense oligonucleotides derived from the mouse and the rat AChE homologous sequences, which have the following sequences:

mEN101 5’-CTGCAATTTCATTCTGACCC-3’ (SEQ ID NO:2)
Griffin and Soreq (1997) Antisense Nucleic Acid Drug Dev. 7(4):351–9
Also referred herein as hEN301.

rEN101 5’-CTGCAATTTCATTCTGACCC-3’ (SEQ ID NO:13)

hEN103 5’-GGGAGAACGAGGAGGACG-3’ (SEQ ID NO:4)

Example 16 demonstrates how administration of mEN101 (EN301) was able to reduce the levels of AChE-R in the brain. This could be done directly, upon crossing the blood-brain-barrier, or indirectly, by reducing the levels of peripheral AChE, increasing the levels of Ach, which would then suppress the production of pro-inflammatory cytokines by macrophages.

Thus, the present invention provides the use of an inhibitor of AChE as defined herein, as a suppressor of pro-inflammatory cytokines release. Known pro-inflammatory cytokines are IL-1β, TNFα, IL-6, IL-8, IL-12 and IL-18, amongst others.

Preferably, IL-1β is the pro-inflammatory cytokine to be suppressed by the method of the invention upon administration of an antisense oligonucleotide denoted by any one of SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:7, or a composition comprising thereof, in a dose effective to reduce the levels of AChE-R in the brain.

Pro-inflammatory cytokine release may be triggered by factors of acquired, chemical or genetic origin. Amongst others, these may be stress, bacterial infection, drugs, irradiation, exposure to AChE inhibitors, stroke, autoimmune diseases, multiple chemical sensitivity, or any cumulative age-dependent damages.

Known conditions which trigger pro-inflammatory cytokine release are: bacterial infection, drugs, irradiation, exposure to AChE inhibitors, stroke, autoimmune diseases, multiple chemical sensitivity, or any cumulative age-dependent damages.

Stress-induced spinal IL-1β over-production and spinal IL-1β suppression following AS-ON inhibition of AChE-R, support the notion of cholineergic regulation of anti-inflammatory response in the CNS. According to this scheme, “stressed” neurons produce high levels of AChE-R, reducing Ach and allowing uninterrupted production of IL-1β in CNS neurons that do not express IL-1β under normal conditions. Antisense suppression of the stress-induced AChE-R would increase ACh levels, which can then suppress IL-1β production in CNS neurons. Such cholinergic regulation of inflammatory response within the CNS may explain both the increase of pro-inflammatory cytokines under cholinergic imbalance (e.g. exposure to organophosphate compounds) [Svensson (2001) ibid.; Dyer (2001) ibid.] and the decrease of those same cytokines under retrieval of cholinergic balance (e.g. under antisense treatment, see FIG. 6). This provides a new understanding of the improvement of survival and clinical status in EAMG rats receiving daily oral doses of EN101 as compared to the conservative AChE inhibitor (pyridostigmine) [Brenner (2003) ibid.].


The antisense oligodeoxynucleotides used as anti-inflammatory agents in the present invention are preferably nuclease resistant. There are a number of modifications that impart nuclease resistance to a given oligonucleotide. Reference is made to WO 98/26062, which publication discloses that oligonucleotides may be made nuclease resistant e.g., by replacing phosphodiester internucleotide bonds with phosphorothioate bonds, replacing the 2'-hydroxy group of one or more nucleotides by 2'-O-methyl groups, or adding a nucleotide sequence capable of forming a loop structure under physiological conditions to the 3' end of the antisense oligonucleotide sequence. An example for a loop forming structure is the sequence 5'-CGGCGAGGCGG-3', which may be added to the 3' end of a given antisense oligonucleotide to impart nuclease resistance thereon.

Phosphorothioate-modified oligonucleotides are generally regarded as safe and free of side effects. The antisense oligonucleotides of the present invention have been found to be effective as partially phosphorothioates and yet more effective as partially 2'-O-methyl protected oligonucleotides. WO 98/26062 teaches that AChE antisense oligonucleotides containing three phosphorothioate bonds out of about twenty internucleotide bonds are generally safe to use in concentrations of between about 1 and 10 μM. However, for long-term applications, oligonucleotides that do not release toxic groups when degraded may be preferred. These include 2'-O-methyl protected oligonucleotides, but not phosphorothioate oligonucleotides. A further advantage of 2'-O-methyl protection over phosphorothioate protection is the reduced amount of oligonucleotide that is required for AChE suppression. This difference is thought to be related to the improved stability of the duplexes obtained when the 2'-O-methyl protected oligonucleotides are used [Lesnik, E. A. & Freier, S. M. (1998) Biochemistry 37, 6991-7]. An alternative explanation for the greater potency of the 2'-O-methyl oligonucleotides is that this modification may facilitate penetration of the oligonucleotide chain through the cell membrane. A further advantage of 2'-O-methyl protection is the better protection against nuclease-mediated degradation
that it confers, thus extending the useful life time of anti-

sense oligonucleotides protected in this way.

[0176] Further, the inhibitor of AChE as defined above
may also be used as an anti-pyretic. Thus, the antisense
oligonucleotides denoted by any one of SEQ ID NO:1, SEQ
ID NO:2 and SEQ ID NO:7, or compositions comprising
thereof, may be used in the method of the invention for
treating fever, or lowering body temperature, in a subject
in need.

[0177] In response to anesthesia, neural regulation induces
rapid decrease in body temperature. As shown in Example
12, transgenic mice with host AChE-R elevation show
inherently higher body temperature as compared to strain,
gender and age-matched controls. Furthermore, their body
temperature remains higher also under anesthesia, demon-
strating impaired regulation and tentative association of
AChE-R with pyrogenic responses. Thus, inhibitors of
AChE-R expression would also have an effect in lowering
the elevated body temperature that is characteristic of
inflammatory reactions.

[0178] Normal body temperature varies by person, age,
activity, and time of day. The average normal body tem-
perature is 37° C. (98.6° F.). An at least half-degree elevation
of the average temperature may already be considered as
fever.

[0179] Fever, or elevated body temperature, may be trig-
gered by various causes, including: viral and bacterial infec-
tions, colds or flu-like illnesses, sore throats and strep throat,
ear infections, viral gastroenteritis or bacterial gastroenter-
itis, acute bronchitis, infectious mononucleosis, urinary tract
infections, upper respiratory infections (such as tonsillitis,
pharyngitis or laryngitis), medications (such as antibiotics,
antihistamines, barbiturates, and drugs for high blood pres-
sure), occasionally, more serious problems like pneumonia,
appendicitis, tuberculosis, and meningitis, collagen vascular
disease, rheumatoid diseases, and autoimmune disorders,
juvener hematoide arthritis, lupus erythematosus, peri- 
arteritis nodosa, AIDS and HIV infection, inflammatory bowel
disease, regional enteritis, ulcerative colitis, cancer, leuke-
emia, neuroblastoma, Hodgkin’s disease and non-Hodgkin’s
lymphoma.

[0180] In accordance with the invention, the dosage of the
antisense oligodeoxynucleotide is about 0.001 to 50 μg
oligonucleotide per gram of body weight of the treated
mammalian subject, and it is for daily use. Preferably, the
dosage is about 0.01 to about 5.0 μg/g. More preferably, the
dosage is between about 0.05 to about 0.7 μg/g. Thus, the
optimal dose range is between 50-500 μg/kg of body weight
of the treated subject, for rats, monkeys and most impor-
tantly humans. This dosage refers to the antisense oligo-
nucleotide administered per se, or in solution, in a pharma-
caceutical composition.

[0181] Further, the present invention also provides a phar-
macological composition for the treatment of conditions
triggering an inflammatory response in a mammalian subject
in need, preferably a human, comprising as active agent
the above-defined inhibitor of AChE expression. Optionally,
the composition further comprises pharmaceutically acceptable
additives, carriers and/or diluents. Preferably, said inhibitor
of AChE expression is an antisense oligonucleotide directed
against AChE, and has the sequence as denoted by any one
of SEQ ID NO:1 and SEQ ID NO:7.

[0182] Alternatively, wherein said mammalian subject is a
non-human mammalian, said antisense nucleotide has the
sequence as denoted by any one of SEQ ID NO:2 and SEQ
ID NO:3.

[0183] In a yet further aspect, the present invention pro-
vides a pharmaceutical composition for the treatment and/or
prevention of inflammation in the joints, central nervous
system, gastrointestinal tract, endocardium, pericardium,
lung, eyes, skin and urogenital system in a mammalian
subject in need, comprising as active agent the inhibitor of
AChE expression as defined above, optionally further compris-
ing pharmaceutically acceptable additives, carriers and/or
diluents. Preferably, said inhibitor of AChE expression is an
antisense oligonucleotide.

[0184] More preferably, wherein said mammalian subject
is a human, said antisense nucleotide has the sequence as
denoted by any one of SEQ ID NO:1 and SEQ ID NO:7.

[0185] Alternatively, wherein said mammalian subject is a
non-human mammalian, said antisense nucleotide has the
sequence as denoted by any one of SEQ ID NO:2 and SEQ
ID NO:3.

[0186] The inhibitor of AChE expression, as defined
above, is to be used in the preparation of the pharmaceutical
composition comprising the same.

[0187] The antisense oligonucleotides described herein
are generally provided in the form of pharmaceutical com-
opositions. Said compositions are for use by injection, topical
administration, or oral uptake.

[0188] Thus, the present invention also provides the use of
the antisense oligonucleotides described herein, and prefer-
ably the use of the antisense oligonucleotides denoted by
SEQ ID NO:1 and SEQ ID NO:7, in the preparation of a
pharmaceutical composition for the treatment or prevention
of conditions triggering an inflammatory response in a
subject in need. In particular, said conditions are selected
from the group comprised of inflammation in the joints,
central nervous system, gastrointestinal tract, endocardium,
pericardium, lung, eyes, skin, urogenital system, fever, the
release of pro-inflammatory cytokines, stroke, brain and
peripheral nerve trauma, neurodegenerative diseases (e.g.
vascular dementia), closed head injury, memory impairment,
and inflammation-associated neuopathies (e.g. Guillain-
Barre syndrome).

[0189] Furthermore, the pharmaceutical composition of
the invention may comprise as active agent a combination of
at least two antisense oligonucleotides as defined in the
invention, or functional analogs, derivatives or fragments
thereof.

[0190] By “analog and derivatives” is meant the “frag-
ments”, “variants”, “analog” or “derivatives” of said
nucleic acid molecule. A “fragment” of a molecule, such as
any of the oligonucleotide sequences of the present inven-
tion, is meant to refer to any nucleotide subset of the
molecule. A “variant” of such molecule is meant to refer a
naturally occurring molecule substantially similar to either
the entire molecule or a fragment thereof. An “analog” of a
molecule can be without limitation a paralogous or ortholo-
gous molecule, e.g. a homologous molecule from the same
species or from different species, respectively.
[0191] Preferred modes of administration of the inhibitor of AChE expression or pharmaceutical compositions comprising the same are by subcutaneous, intraperitoneal, intravenous, intramuscular or systemic injection.

[0192] The pharmaceutical composition described herein generally comprises a buffering agent, an agent which adjusts the osmolarity thereof, and optionally, one or more carriers, excipients and/or additives as known in the art, e.g., for the purposes of adding flavors, colors, lubrication, or the like to the pharmaceutical composition.

[0193] A preferred buffering agent is Tris, consisting of 10 mM Tris, pH 7.5-8.0, which solution is also adjusted for osmolarity.

[0194] For in vivo use, the antisense oligonucleotides are suspended is sterile distilled water or in sterile saline.

[0195] Other carriers may include starch and derivatives thereof, cellulose and derivatives thereof, e.g., microcrystalline cellulose, xanthan gum, and the like. Lubricants may include hydrogenated castor oil and the like.

[0196] Topical administration of pharmaceutical compositions may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

[0197] Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets or tablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable.

[0198] Compositions and formulations for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

[0199] The pharmaceutical compositions described herein include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions may be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids.

[0200] The pharmaceutical compositions of the present invention, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product. Such compositions may be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

[0201] The pharmaceutical compositions may be formulated and used as foams. Pharmaceutical foams include formulations such as, but not limited to, emulsions, microemulsions, creams, jellies and liposomes. While basically similar in nature these formulations vary in the components and the consistency of the final product.

[0202] In one embodiment, the pharmaceutical composition of the invention is for daily use by a subject in need of such treatment, at a dosage of active ingredient between about 0.001 μg/g and about 50 μg/g. Preferably, the treatment and/or prevention comprises administering a dosage of active ingredient of about 0.01 to about 5.0 μg/g. Most preferably, said dosage of active ingredient is of between about 0.05 to about 0.70 μg/g, and even most preferably, the dosage is from 0.15 to 0.50 μg/g of body weight of the subject in need.

[0203] Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the antisense oligonucleotide in bodily fluids or tissues. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 0.01 μg/g to 100 μg/g of body weight, once or more daily, to once every 20 years.

[0204] The preparation of pharmaceutical compositions is well known in the art and has been described in many articles and textbooks, see e.g., Gennaro A. R. ed. (1999) Remington’s Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., and especially pages 1521-1712 therein.

[0205] The results presented herein are the first demonstration of an organismal antisense response that affects primate CNS neurotransmission (Example 1). Positron Emission Tomography (PET) imaging studies in Rhesus monkeys demonstrated for 2'-O-methylated oligonucleotides limited, yet relatively long-term persistence in the brain as compared with phosphothioate agents [Tavittian (1998) id. ibid.]. In addition, the blood-brain barrier of primates may be more easily penetrated than that of rodents, which is compatible with the inventors’ recent findings [Tomkins, O. et al. (2001) Cell Mol Neurobiol 21: 675-691].

[0206] The antisense agent targeted toward the human AChE sequence (see Examples) appeared effective in Cynomolgus monkeys at the same nanomolar dose as that of the corresponding agents in mice [Cohen et al. (2002) id. ibid.] and rats Brenner et al. (2003) id. ibid.]. Long-term AChE-R overproduction, as is the case in head-injured mice, is associated with impaired locomotion control that is susceptible to improvement under antisense suppression of AChE-R production [Shohami (2000) id. ibid.]. In spite of the limited number of experimental animals used in the current study, delivery was appeared to be effective in both the intravenous and the oral administration mode, with dose dependence reflected by the more pronounced effects under 500 as compared to 150 μg/kg/day of orally administrated hEN101.

[0207] In conclusion, the present invention teaches methods of treatment of conditions wherein lowering the amounts
of circulating AChE-R may be therapeutic and even preventive. Mainly, said conditions may be summarized as conditions triggering an inflammatory response, inflammation of any kind, and in particular inflammation-associated neuropathies, such as Guillain-Barré syndrome. The method comprises administering a therapeutically effective amount of an inhibitor of AChE expression or a composition comprising the same to a mammalian subject in need, preferably a human.

[0208] Preferably, said inhibitor of AChE expression to be used in the methods of the invention is an antisense oligonucleotide, which, more preferably has the sequence as denoted by any one of SEQ ID NO:1 and SEQ ID NO:7.

[0209] Said therapeutic effective amount, or dosing, is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual oligonucleotides, and can generally be estimated based on EC_{50} found to be effective in in vitro as well as in vivo.


[0211] The methods described herein also include combination therapy, where the inhibitor of AChE expression or the composition comprising thereof are administered in combination with other drugs, in accordance with the condition of the subject to be treated.

[0212] As shown in Examples 6-11, administration of a low dose of endotoxin to healthy volunteers induces secretion of pro-inflammatory cytokines and cortisol, compromises cholinergic homeostasis and alters memory. Both psychological [Maes M. et al. (1998) Cytokine 10:313-8], and physical [Goodman J. C. et al. (1990) J. Neuroimmunol. 30:213-7] stressors are likewise associated with the production of pro-inflammatory cytokines (including TNF-α and IL-6) in humans. Exposure to stressful stimuli exerts profound effects on cholinergic homeostasis in general and on the production and cellular distribution of AChE-R in particular. Therefore, experimental endotoxemia emerges as a valid model for studying the interactions between cytokines and the changes in cholinergic homeostasis (as those are reflected by AChE-R modulations) as well as the impact of these interactions on memory functions. No subjective feelings of illness were involved, so that the endotoxin-induced memory alterations could not be attributed to a perceived physical-illness-associated distress. The selectivity of the observed memory changes was compatible with reports by others that cortisol does not affect attention, verbal executive function or vigilance [Lupien et al. (1999) Rev. Neurosci. 10: 117-39].

[0213] FIG. 11 presents a scheme summarizing the kinetic follow-up for the different parameters that were measured and the postulated associations between them, predicting potentially causal relationships between the induction of cytokines, hormone secretion, AChE modulations and the resultant memory changes. Interestingly, during the first testing period the endotoxin-induced impairment in declarative memory was highest and correlated positively with cytokine secretion, whereas the improvement in working memory became prominent at 3 hr post-treatment and showed no correlation with cytokine secretion. In contrast, both types of memory changes were significantly correlated with AChE-R cleavage, although cholinergic control over working memory seemed to begin earlier than for declarative memory (3 hr vs. 9 hr post-injection, FIG. 11B and FIG. 11C, respectively).

[0214] Previous reports have documented decrements in declarative memory following endotoxin administration to healthy volunteers [Reichenberg (2001) id ibid.], as well as following cytokine (especially interleukin and interleukin-2) therapy [Meyers C. A. (1999) Adv. Exp. Med. Biol. 461:75-81; Capuron L. et al. (2001) Psychosom. Med. 63:376-86], viral (e.g., influenza) infection [Capuron (1999) id ibid.] or cortisol administration [de Quervain, D. J. et al. (2000) Nat. Neurosci. 3:313-4]. In this study, the endotoxin-induced decrease in declarative memory performance was associated with cytokines secretion only in the first testing period. In contrast, it was associated with AChE activity and AChE-R cleavage levels during the last period, when cytokine concentrations have returned to baseline yet the differences between AChE activity and AChE-R cleavage were maximal between the endotoxin and the placebo conditions. These findings may suggest that immune-mediated processes are prominent in the early endotoxin-induced memory impairments, whereas the later effects are probably mediated by the cholinergic system.

[0215] This study demonstrates that changes in memory functioning following endotoxin exposure are co-associated with the induction of pro-inflammatory cytokines and AChE-R cleavage. The tentative pathway through which these changes may occur involves alterations in cholinergic neurotransmission and elevation in cytokine secretion (FIG. 11). These are associated with many medical conditions that involve inflammatory processes, particularly within the brain (e.g., stroke, brain trauma and neurodegenerative disease, such as vascular dementia) [McGeer P. L. and McGeer E. G. (1995) Brain Res. Rev. 21:195-218; Saito H. et al. (1995) Clin. Exp. Pharmacol. Physiol. Suppl. 22:S257-9; Levin and Simon (1998) id ibid.]. For example, closed head injury effects in the production of TNF-α and other pro-inflammatory cytokines [Goodman et al. (1990) id ibid.; Tremblay V. et al. (1999) J. Interferon Cytokine Res. 19:791-5] as well as in excessive accumulation of AChE-R within the brain [Slohan et al. (2000) id ibid.]. The findings presented herein suggest that cytokine-cholinergic interactions play an important role in the memory alterations that
accompanied these conditions, and may provide insights into the development of novel preventive and therapeutic procedures that will counteract the corresponding memory impairments without harming the improved capacities.

[0216] Disclosed and described, it is to be understood that this invention is not limited to the particular examples, process steps, and materials disclosed herein as such process steps and materials may vary somewhat. It is also to be understood that the terminology used herein is used for the purpose of describing particular embodiments only and not intended to be limiting since the scope of the present invention will be limited only by the appended claims and equivalents thereof.

[0217] It must be noted that, as used in this specification and the appended claims, the singular forms “a”, “an” and “the” include plural referents unless the context clearly dictates otherwise.

[0218] Throughout this specification and the claims which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising”, will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[0219] The following Examples are representative of techniques employed by the inventors in carrying out aspects of the present invention. It should be appreciated that while these techniques are exemplary of preferred embodiments for the practice of the invention, those of skill in the art, in light of the present disclosure, will recognize that numerous modifications can be made without departing from the spirit and intended scope of the invention.

EXAMPLES

[0220] The basic working hypothesis guiding this study was that stimulus-induced modulations in the levels and composition of neuronal AChE variants, CHAT and VACH/T together contribute toward the maintenance of cholinergic homeostasis in primate motorneurons. This predicted neuronal AChE-R overproduction as well as CHAT and VACH/T suppression under handling stresses and, if so, whether antisense suppression of AChE-R would attenuate neuronal IL-1β accumulation.

Experimental Procedures

Experimental Procedures Employed in Studying the Anti-Inflammatory Effects of hEN101 in the Primate Spinal Cord

[0221] Animals: 15 month-old purpose-bred cynomolgus monkeys were supplied by Charles River (UK) Ltd. Anti-sense administration was performed at Huntington Life Sciences Ltd. (Huntingdon, UK), in compliance with all of the relevant regulations for animal experimentation in the UK.

[0222] Test substance: Human (h) HPLC-purified, GLP grade EN101 (purity 95% as verified by capillary electrophoresis) was purchased from Avevia Biotechnology (Milford, Mass.). The primary hEN101 sequence, 5’CTGCCACGTCTCTGGCA*G*C*G*3’ (SEQ ID NO:1), is complementary to the coding sequence of human AChE mRNA (GeneBank Accession No. NM 000665, nucleotide positions 733-752) within exon 2, common to all three AChE variants [Soreq, H. & Zakut, H. (1993) Human cholinesterases and anticholinesterases, Academic Press, INC., San Diego; Ben-Azir-Aloya, R. et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 2471-2475]. The three 3'-terminal residues (*) were protected against nuclease attack with oxyethyl groups at the 2' position. The sequence representing hEN101 with the three 3'-terminal bases modified is denoted by SEQ ID NO:7. Lyophylized oligonucleotides were reconstituted in sterile double distilled water (24 mg/ml), and stored at -20°C.


[0224] hEN101 stability: Stability of freeze-dried hEN101 was tested by HPLC during storage at ~20±5°C, 4°C and 25±2°C (60±5% relative humidity) in the dark. Three samples from each storage condition were collected after 3, 6 and 9 months and their stability analyzed by HPLC. hEN101 was found to be stable for at least 6 months at ~20°C under these storage conditions.

[0225] hEN101 administration: Three pairs of 1.5 to 2.5 Kg cynomolgus monkeys, 1 male and 1 female, were administered hEN101 for 7 days: 150 µg/kg daily per os (p.o.) by oral gavage (15 µg/ml in 0.9% saline) or 500 µg/kg daily (p.o., 50 µg/ml in saline) or by intravenous (i.v.) injection (100 µg/ml in saline). Plasma samples were
removed at the noted hours following the second day of treatment and kept at -20°C until use. Following 1 week of daily treatment, animals were euthanized and lumbar spinal cord preparations were paraffin-embedded by standard procedures. One male naïve monkey served as control.

**[0226]** Toxicology: Potential toxicity of hEN101 was tested at Huntington before, during and following treatment. Among the parameters noted were body weight, food consumption, general locomotor behavior, electrocardiography and blood pressure, blood count, prothrombin time and standard blood chemistry (Hitachi 917 Clinical Chemistry Analyzer). Post-mortem observation included organ weights and scanning of hematoxylin and eosin-stained sections of brain, heart, kidneys, liver, lungs, spinal cord and stomach.

**[0227]** In situ hybridization: Tissues were fixed in 4% paraformaldehyde and cut into 7 μm paraffin-embedded sections. Lumbar spinal cord sections were deparaffinized, rehydrated using serial ethanol dilutions and permeabilized with proteinase K (10 μg/ml, 10 min at 37°C). Slides were exposed to 5' biotinylated, fully 2’-oxymethylated AChE-R or AChE-S-specific 50-mer cRNA probes complementary to human AChE pseudointron 4 or exon 6, respectively (Microsynth, Bielgach, Switzerland). The following probes were employed:

**[0228]** human AChE-R probe (nucleotide positions 88-38 in GenBank Accession No. S 71129; SEQ ID NO:5):

5’-CUAGGGGGAGAAGAGAGGGGUUACACUGGCGGGCUCCCACUCCCCUCUC-3’

**[0229]** human AChE-S probe (nucleotide positions 2071-2022 in GenBank Accession No. NM 000665; SEQ ID NO:6):

5’-CCGGGGGACGUCGGGGUGGGGUGGGGAUGGGCAUGUGGGUCUGUCU-3’.

**[0230]** Hybridization was performed overnight at 52°C in hybridization mixture containing 10 μg/ml probe, 50 μg/ml yeast tRNA, 50 μg/ml heparin and 50% formamide in 375 mM Na chloride, 37.5 mM Na citrate, pH 4.5. Slides were washed to remove unhybridized probe, blocked with 1% skim milk containing 0.01% Tween-20 and 2 mM levamisol, an alkaline phosphatase inhibitor used to suppress non-specific staining and incubated with streptavidin-alkaline phosphatase (Amersham Pharmacia, Little Chalfont Bucks, UK). Fast Red™ substrate (Roche Diagnostics, Mannheim, Germany) was used for detection.

**[0231]** Immunohistochemistry: Re-hydrated spinal cord sections were subjected to heat-induced antigen retrieval by microwave treatment in 0.01 M citrate buffer, pH 6.0. Non-specific binding was blocked by 4% naïve goat or donkey serum in PBS with 0.3% Triton X-100 and 0.05% Tween20™. Slides were incubated with primary antibodies diluted in the same buffer (1 h, room temp., overnight, 4°C). Sections were rinsed and incubated with biotin-conjugated secondary antibody, diluted (1:200) in the same blocking buffer (3 h, room temp.). The primary antibodies included rabbit polyclonal anti-VAChT (1:100, Sigma, St. Louis, Mo.), goat polyclonal anti-ChAT (1:50, Chemicon International, Temecula, Calif.) and goat anti-IL-1β (1:20, R&D systems, Minneapolis, Minn.). Biotinylated secondary antibodies were donkey anti-rabbit (Chemicon) and donkey anti-goat (Jackson ImmunoResearch Laboratories, West Grove, Pa.), both used at 1:200 dilutions. Detection was with Fast Red™ substrate for anti-VAChT and ChAT antibodies and with Vectastain ABC peroxidase kit (Vector Laboratories, Burlingame, Calif.) for the anti-IL-1β antibody.

**[0232]** Confocal microscopy: was carried out using a Bio-Rad MRC 1024 confocal scanner (Hemipinsteds, Hertfordshire, U.K.) coupled to an inverted Zeiss Axiovert 135 microscope (Oberkochen, Germany) equipped with a Plan Apochromat 40×1.3 immersion objective. Fast Red was excited at 488 nm and emission was measured through a 590DF52 interference filter (580±16 nm). Immunolabeled sections were scanned every 0.5 μm and projections analyzed using the Image Pro Plus 4.0 (Media Cybernetics, Silver Spring, Md.) software.

**[0233]** Cholinesterase activity measurements: Plasma samples were subjected to cholinesterase catalytic activity measurements [Ellison, G. L. et al. (1961) Biochem. Pharmacol. 7, 88-99] adapted to a multi-well plate reader. Acetylthiocholine (ATCh) hydrolysis rates were measured following prior incubation for 30 min with 5x10^-7 M of the specific butryrylcholinesterase (BuChE) inhibitor tetraiso-propylpyrophosphoramide, iso-OMPA. Total plasma cholinesterase activities were measured in the absence of inhibitors.

Experimental Procedures Employed in Studying the Relationship Between AChE-R, Cytokines and Memory

**[0234]** Subjects of the memory study: Ten male subjects participated in the study, which was approved by an independent ethics committee. Subjects recruitment as well as physical and psychiatric screening, were described in detail elsewhere [Reichenberg A. et al. (2001) id ibid.]. The current study involved a subset of the subjects included in the previous project, with serum AChE and working memory tests added. Interviews by experienced psychiatrists excluded the presence and the history of any axis I psychiatric disorder according to the DSM-IV [American Psychiatric Association (1994) Diagnostic and statistical manual for mental disorders, 4th ed., Washington D.C.]. Only subjects who successfully passed the screening procedure, and signed an informed consent form, were considered eligible to participate. Comprehensive assessment was performed, and involved each subject going through a number of physical and neuropsychological tests in a clinical research unit using a balanced, randomized, double-blind, cross-over design.

**[0235]** Procedure for the memory tests: All technical equipment, including the blood sampling device, was housed in a room adjacent to the sound-shielded experimental room. Every subject passed two 10 days apart testing sessions and spent the night before each experimental session in the research unit. A battery of neuropsychological tests, assessing memory, learning, and attention was given for adaptation upon their first arrival in the evening, minimizing subsequent practice effects [McCaffrey, R. J. and Lynch, J. K. (1992) Neuropsychol. Rev. 3:235-48]. Alternate versions of these tests were used in the experimental testing sessions. In the next morning, an intravenous cannula was
inserted into an antecubital forearm vein for intermittent blood sampling and intravenous (i.v.) injection of endotoxin (0.8 ng *Salmonella abortus equi* endotoxin per Kg body weight) in one session or the same volume of 0.9% NaCl (saline) solution on the other occasion (placebo). The order of injections was balanced, so that half of the subjects received the saline injection and half received the endotoxin injection first. No significant differences were found between the groups defined by the treatment order in either age, years of education, or body weight. The experimenters and the subject were blind with respect to the group assignment. During each session, subjects were tested three times, at 1-2, 3-4 and 9-10 hr post-injection. Blood was collected at baseline before i.v. injection, and at the beginning of each testing period. Rectal temperature was measured continuously using a thermistor probe. Self-reported physical sickness symptoms (headaches, muscle pain, shivering, nausea, breathing difficulties, and fatigue) were assessed at the end of each testing period, by a questionnaire using a 5-point Leikart scale (0—no symptoms, 4—very severe symptoms).

[0236] *Salmonella abortus equi* endotoxin: Prepared for use in humans, this endotoxin was available as a sterile solution free of proteins and nucleic acids. The endotoxin preparation employed has proven to be safe in various studies of other groups [Burrell R. (1994) id ibid.] and in studies at the Max Planck Institute of Psychiatry, including more than 100 subjects since 1991 [Pollmacher T. et al., (1996) J. Infect. Dis. 174:1040-5].

[0237] Plasma levels of AChE and its degradation product, cytokines and cortisol: Blood was collected in tubes containing Na-EDTA and aprotinin and was immediately centrifuged. Plasma was aliquoted and frozen to −80°C. AChE catalytic activity was measured as the capacity for acetylcholine (ATCh) hydrolysis in the presence of 1×10−5 M tetraisopropylpyrophosphoramide (iso-OMP), a selective inhibitor of serum butyrylcholinesterase, BChE [Soroq H. and Glick D. (2000): Novel roles for cholinesterases in stress and inhibitor responses. In: Giacobini E. (ed.) Cholinesterases and Cholinesterase Inhibitors: Basic, Preclinical and Clinical Aspects. London, Martin Dunitz, pp 47-61]. Endotoxin-induced differences were calculated by subtracting activities in the absence of endotoxin, with each individual serving as its own control and daily hour carefully matched. To evaluate AChE-R concentrations and integrity, plasma proteins (40 μg) were subjected to 4-20% polyacrylamide gel electrophoresis under fully denaturing conditions (BioRad Laboratories, Hercules, Calif.), blotted to nitrocellulose filters, incubated with rabbit anti-AChE-R antibodies [Streufeld M. et al. (2000) Proc. Natl. Acad. Sci. USA 97:8647-8652] and peroxidase-conjugated anti-rabbit immunoglobulins, and subjected to ECL™ detection (Amer sham Pharmacia Biotech, UK), densitometric analysis and quantification as described [Shohami (2000) id ibid.]. The plasma levels of cortisol were determined by a radioimmunoassay, and the plasma levels of cytokines and soluble cytokine receptors were assessed by commercial enzyme-linked immunosorbent assays [Mullington J. et al. (2000) Am. J. Physiol. Regul. Integr. Comp. Physiol. 278:R947-55]. Labeling AChE-R mRNA and its protein product in vascular endothelial cells: Fluorescent in situ hybridization and immunohistochemistry of AChE-R mRNA and AChE-R protein were performed and quantified as reported [Cohen (2002) id ibid.; Perry, C. et al. (2002) Oncogene 21:8428-8441] using paraffin-embedded tissue sections from surgically-removed biopsies of patients with or without clinical inflammation due to non-specific kidney vasculitis or following kidney rejection.

[0238] MALDI-TOF-MS analysis of immunolabeled proteins: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) was employed in an attempt to identify the protein and peptide bands labeled by anti-AChE-R antibodies in blotted membranes. Proteolytic degradation of the gel-eluted peptide was performed using the endoprotease LysC from *Achromobacter*lyticus (Wako Chemicals, Inc., USA) at a substrate to enzyme ratio of 200:1. Digestion was carried out overnight in 0.05M Tris HCl, pH 9.0, in the presence of 4M urea, at 30°C.

[0239] Neuropsychological assessment: Declarative memory was assessed using the Story Recall test [Green P. and Allen L. M. (1995): *Manual for the CogniSys Story Recall test* CogniSyst Inc., Durham, N.C.]. Subjects were requested to repeat a 25-item story from memory immediately, and 30 min after presentation. The total number of correct verbatim recall was counted. Memory span and working memory were assessed using the Digit Span forward & backward [Wechsler D. (1987): *Wechsler Memory Scale, Revised Manual* The Psychological Corp. San Antonio, Tex.]. Subjects were requested to repeat lists of digits with increased number of digits every two lists either in the correct order of presentation (forward condition—assessment of span), or in a reversed order (backward condition—assessment of working memory). The number of lists correctly repeated was counted. Attention was assessed using the Ruff &T. cancellation test [Ruff R. M. and Allen C. C. (1996): *Ruff &T Selective Attention Test: Professional Manual*. Psychological Assessment Resources Inc., Lutz, Fla.]: Subjects were instructed to mark either the digit 2 or the digit 7, which are randomly placed either between letters or between digits. The numbers of correct responses in a 5 minute trial were counted.

[0240] Statistical analyses: The main hypotheses concerning treatment effects on AChE activity, AChE-R levels, and neuropsychological performance were tested using repeated measure analysis of variance models (ANOVARs). Repeated measure ANOVAs were also used to examine the treatment effect on physical sickness symptoms, on plasma levels of cytokines and cortisol and on body temperature. The level of significance was set at the critical value of p<0.05 (two tailed). Whenever significant treatment-by-time interactions were found, the simple effects were analyzed as suggested [Winer B. et al. (1991): *Statistical Principles in Experimental Design*. 3rd ed. McGraw-Hill, New York], and Tukey’s adjustments were applied.

[0241] To assess the associations between changes from the placebo to the endotoxin condition in AChE activity, AChE-R levels, and physiologial (cytokines and cortisol secretion), and neuropsychological parameters, Pearson’s correlation coefficients were calculated.

[0242] No deviation from normal distributions was evident for any of the dependent variables. No univariate outliers were found using Z-scores and no multivariate outliers were found using the Mahalanobis distance [Tabachnick B. G. and Fidell L. S. (2001) *Using Multivariate Statistics*. 4th ed. Allyn and Bacon, Boston, Mass.]. To adjust for any non-homogeneity of covariance for the
within-subject effects, we used p values that were adjusted using the Huynh-Feldt method [Norusis M. J. (1994) SPSS advanced statistics 6.1. SPSS Inc., Chicago, Ill.]. Analyses were carried out using SPSS 10.

[0243] Linear rank Wilcoxon test for two related samples was used for the analysis of AChE-R- and IL-1β-positive fractions of analyzed neurons, measured on at least 4 sections from each group. Differences were considered significant when a p value of ~0.05 or less was obtained using the SAS 8.0 software. Student’s t test was used for analyzing the numbers and volume of VACHt-containing terminals in spinal cord sections.

Experimental Procedures Employed with the GBS Model

[0244] Pre-treatment, sensitization and LPS exposure: Systemic Campylobacter or E. coli LPS exposure was done as previously described [Iergane G. et al., (2003) J. Neurol. Sci. 213: 11-14]: Female 8-week-old Lewis rats were sensitized with 100 g KLLH by subcutaneous (s.c.) injections administered to the base of tail on days 1 and 21 followed by intraperitoneal injection of 15 microgram Cj 0:19 or E. Coli 055:B5 LPS on day 28. In vitro LPS exposure: Lewis rats were similarly sensitized with KLLH. On day 28, the rats were anaesthetized, their spleens removed and disintegrated into cell suspension. The cells were suspended in RPMI-1640 medium containing antibiotics and glutamine. Following centrifugation, the pellet was resuspended in RPMI, layered on Histopaque and centrifuged again. The lymphocyte fraction was collected, washed and supplemented with fetal calf serum and diluted to a concentration of 4.5x10⁷ cells/ml. Splenocytes reacted with LPS additionally contained 0.5 mg/ml Cj-LPS. Following incubation for 48 hour at 37° C, with 5% CO₂, the cell suspension was centrifuged and supernatant medium collected and stored at ~20° C. until use.

[0245] Bone-marrow derived macrophages (BMM): Rat femur marrow content was obtained as described elsewhere [Apte, R. N., and Keisari, Y. (1987) Immunobiology 175: 470-481], dispersed into RPMI 1640 medium, washed, supplemented with serum and L-cell conditioned medium as a source of a colony stimulating factor and cultured at 37° C. 5% CO₂. After 7 days a macrophage monolayer was harvested.

[0246] Intraneural injection: Female 8-week-old Lewis rats were anesthetized by intraperitoneal injection of 10% solution of chloralhydrate (0.3-1 ml). The sciatic nerves exposed at the mid-thigh through a skin incision from the sciatic notch to the popliteal fossa. Tested mediums or solutions, 10 µl each, were intraneurally injected to separate sciatic nerves, via hand held Hamilton microsyringe with a 30 gauge needle under a dissection microscope.

[0247] Electrophysiological assessment was done as we previously described [Iergane (2003) id ibid.]: Nerve conduction was performed prior to, 10 minutes, 1, 2, 3, 4 and 7 days following intraneural injection under general anesthesia by chloralhydrate solution of (0.3-1 ml) at room temperature. The sciatic nerve was supramaximally stimulated at the sciatic notch, and the peroneal and posterior tibial nerves at the ankle via needle electrodes. Compound muscle action potentials (CMAP) was recorded from the intrinsic foot muscles (both extensor digitorum brevis and flexor digitorum brevis). CMAPs, their baseline to peak amplitude, latency and duration were measured and the proximal and distal amplitudes ratio (PDR) calculated for each nerve. A PDR of less than 0.5 was considered a conduction block.

[0248] Tissue preparations: For western blot analysis, 7 µm of the sciatic nerve including the injection site were removed under general anesthesia as described, quickly frozen in liquid nitrogen, and stored at ~70° C. For morphological analysis, the nerve segments were immersed in 4% paraformaldehyde in PBS (48 hrs, 4° C.), embedded in paraffin and sectioned at 8 µm in the axial or longitudinal planes.

[0249] In situ hybridization and immunohistochemistry. As previously reported [Dori A. et al., (2005) Cereb Cortex 15(4): 419-30], sections were deparaffinized, rehydrated and boiled in a microwave (750 W, 15 min) in 0.1M citric buffer (pH 6.0). Cy5-conjugated streptavidin and Cy3-conjugated anti-digoxigenin will be employed for detection of AChE-R mRNA specific biotin- and AChE-S mRNA specific digoxigenin-labeled probes, respectively. Cy3- or biotin-conjugated secondary IgG reacted with avidin-bound peroxidase-complex (ABC Elite, Vector Laboratories) will be employed for detection of AChE-R mRNA. AChE immuno-staining will be performed by light microscopy following peroxidase reaction, respectively. Selected sections will be counterstained with Gill-2 hematoxylin.

[0250] Image analysis: Confocal microscopy and Scion Image software (Scion Corporation, Frederick, Md.) will be applied as described [Dori (2005) id ibid.].

[0251] Immunoblot: Nerve homogenates were produced by grinding with a pestle and mortar and processed as we previously described [Dori (2005) id ibid.].

[0252] Catalytic activity Acetylthiocholine hydrolysis will be measured spectrophotometrically as described [Kaufer (1998) id ibid.], using Iso-OMPA (tetraisopropylpyrophosphoramide) to block butryrylcholinesterase activity (S-10-M).

[0253] Statistical analysis: ANOVA (Statistica software, StatSoft, Tulsa, Okla.) will be used to compare multiple groups and one-tailed t-test (Microsoft Excel) to compare two groups.

Example 1

Treatment-Reduced VACHt and ChAT Labeling in Spinal Cord Motoneurons

[0254] VACHt was predictably concentrated in cholinergic (C) terminals surrounding motoneurons [Weishe (1996) id ibid.], where it loads neural vesicles with ACh. Confocal microscopy projections of spinal cord motoneurons (cell diameter ~40 µm) from hEN101-treated monkeys as compared with the naive state showed small but significant dose-independent decreases (p<0.01, Student’s t test) in the average number of VACHt-positive C-terminals per cell (FIGS. 1A, 1B), suggesting a handling stress effect on loading C-terminals with ACh. VACHt-Labeled C-terminals were significantly smaller (<60 µm²) under p.o. administration of 150 µg/kg/day as compared to control sections (FIGS. 1B and 1C, p<0.01, Student’s t test), perhaps reflecting changes in VACHt translocation into vesicles and/or VACHt stability.

[0255] VACHt production is largely co-regulated with that of ChAT [Udlin, T. B. et al. (1995) Trends Neurosci. 18, 218-224], since both are produced from one gene complex (the so-called “cholinergic locus”) [Ericson, J. D. et al. (1996) Prog. Brain Res. 109, 69-82]. ChAT staining of C-terminals on motoneurons indeed presented similar changes to those observed for VACHt staining (data not
shown). In addition, anti-ChAT antibodies labeled in control sections several partition cells (FIG. 1D), from which cholinergic terminals emerge to motoneurons [Barber, R. P. et al. (1984) J. Comp. Neurol. 229, 329-346]. Lumbar spinal cord sections from hEN101-treated monkeys, regardless of the dose or mode of administration, revealed conversely decreased staining intensity of ChAT-positive partition cells (FIG. 1E), again indicating handling stress-related suppression of ACh production and slowdown of vesicle recycling.

Example 2

[0256] EN101 Prevention of Stress-Induced Increases in Plasma AChE Activity

Cholinesterase activities were measured in plasma samples taken during the second day of hEN101 administration. ATCh hydrolysis in plasma is largely due to serum BuChE, the primary cholinesterase encoded by a non-homologous mRNA, which remained generally unchanged. However, plasma also included a minor, but significant AChE activity [Zakut, H. et al. (1998) Cancer 61, 727-737], measurable following pre-incubation in the presence of 5x10^(-5)M of the BuChE-specific inhibitor, iso-OMPA. AChE activity increased, as compared with the values before treatment (pre-dose), within the 5 hr following the stressful oral gavage administration of 150 μg/kg EN101 (Table 1), potentially reflecting increased production under handling. This further indicates a short half-life for primate AChE-R mRNA in vivo, compatible with previous findings [Chan (1991) id ibid; Brenner et al. (2003) id ibid.]. Increases were effectively suppressed by the higher oral dose of 500 μg/kg EN101, and yet more so following i.v. of administration of 500 μg/kg EN101 (Table 1), possibly reflecting dose-dependent hEN101 prevention of AChE-R synthesis.

**TABLE 1**

<table>
<thead>
<tr>
<th>hEN101 dose (μg/kg)</th>
<th>Mode of administration</th>
<th>0 hr post-treatment</th>
<th>p.o. 150</th>
<th>p.o. 500</th>
<th>i.v. 500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ChE activity</td>
<td>0.100 ± 0.010</td>
<td>0.100 ± 0.010</td>
<td>0.100 ± 0.010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(% of pre-treatment)</td>
<td>3.0 ± 0.3</td>
<td>1.0 ± 0.1</td>
<td>0.4 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AChE activity</td>
<td>0.96 ± 0.025</td>
<td>0.96 ± 0.025</td>
<td>0.96 ± 0.025</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(% of pre-treatment)</td>
<td>12.0 ± 0.4</td>
<td>12.0 ± 0.4</td>
<td>12.0 ± 0.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1Percent changes in the ATCh hydrolysis rates in plasma samples from monkeys treated twice on 2 consecutive days with the noted amounts and administration routes of hEN101.
2In the absence of inhibitors, hydrolysis rates reflect activity of the abundant cholinesterase in plasma, BuChE.
3AChE specific activity, measured in the presence of 5 x 10^(-5) M of the specific BuChE inhibitor, iso-OMPA. Values represent average ± SEM from six measurements in plasma samples derived from 2 monkeys. Mean AChE and BuChE absolute activity.

Example 3

EN101 Effects on AChE-R and AChE-S mRNAs in Monkey Spinal Cord Neurons

[0258] Paraffin-embedded sections of lumbar spinal cord from Cynomolgus monkeys treated for 7 days once daily with hEN101 were subjected to high resolution fluorescent in situ hybridization (FISH). Variant-specific FISH probes (FIG. 2A) revealed AChE-S more than AChE-R mRNA labeling in numerous punctate areas and longitudinal threads, possibly cross-sections and longitudinal sections through neuronal processes (FIG. 2B-2C). This difference, albeit statistically non-significant was compatible with previous observations demonstrating AChE-S, but not AChE-R mRNA in murine neuronal processes under normal conditions [Meshorer (2002) id ibid.]. The higher oral and i.v. dose yielded reduced AChE-R mRNA labeling (FIGS. 2G and 2I) as compared to the lower dose, FIG. 2E). AChE-S mRNA-labeled neurons displayed limited EN101-induced suppression (FIG. 2H, 2J as compared to 2D), with reduced process labeling (FIGS. 2F, 2H and 2J). Positron Emission Tomography (PET) imaging studies in Rhesus monkeys demonstrated for 2’-O-methylated oligonucleotides limited, yet relatively efficient penetration to the brain as compared with phosphorothioate agents [Tavtian et al. (1998) id ibid.]. In addition, the blood-brain-barrier of primates may be more easily penetrated than that of rodents [Tomkins et al. (2001) Cell Mol. Neurobiol. 21: 675-91]. Nevertheless, this is the first demonstration of an organismal antisense response that affects primate CNS neurons.

[0259] At the same nanomolar dose as that of the corresponding agents in mice [Cohen (2002) id ibid.], and rats [Brenner (2003) id ibid.], delivery of human EN101 appeared in Cynomolgus monkeys to be effective in both the intravenous and the oral administration mode, as it did in rats [Brenner (2003) id ibid.]. Albeit in a limited number of animals, dose dependence was reflected by the more pronounced effects under 500 as compared to 150 μg/kg/day of orally administered hEN101.

Example 4

Antisense Destruction of AChE-R mRNA is Inversely Related to Perikaryon Size

[0260] Similarly sized neurons in hematoxylin-eosin stained spinal cord sections (FIG. 3A) were sorted into three size groups according to their cell body diameter (FIG. 3B): motoneurons (<40 μm, 20-35% of total counted neurons, localized to motor nuclei in the ventral horn and intermediate zone), medium-sized neurons (20-40 μm, almost 60%, dispersed throughout the spinal cord, mainly in the ventral horn and intermediate zone), and small neurons (10-20 μm, 5-20%, located primarily in the dorsal horn). AChE-S and AChE-R mRNA labeled cell fractions from each group were evaluated in adjacent sections of small and medium sized AChE-R positive cells (<40 μm diameter) by over 4-fold as compared to the native state (p=0.057 for small cells, Wilcoxon test). AChE-R-positive smaller neuron fractions dropped significantly under the higher hEN101 oral dose (p=0.033, Wilcoxon test), compared to the 150 μg/kg/day treatment, and even further under its i.v. administration (p=0.015). Medium sized fractions dropped significantly following i.v. 150 μg/kg/day as compared to p.o. administration of 150 μg/kg/day (p=0.030). Reduced staining intensity suggested a certain antisense effect in motoneurons, as well, albeit with relatively limited efficacy. However, there was no discernable reduction in the total fractions of labeled large cell bodies by any treatment (p=0.100). This possibly reflects distinct membrane and/or metabolic properties, dif-
ifferent cell volumes or a combined contribution of these properties. For AChE-S mRNA, the number of large positive cell bodies remaining unchanged, whereas positive small and medium sized neurons, were reduced by 50% and 20%, respectively under either low or high dose of hEN101 as compared to naïve. The apparent dose-independence of changes in AChE-S mRNA is compatible with the hypothesis that these changes were not nosense driven, but could possibly reflect the effect of handling stress of shifting splicing from AChE-S to AChE-R [Kaufler (1998) id ibid.].

**Example 5**

hEN101 Suppression of Neuronal Pro-Inflammatory Cytokines

[0261] Lumbar sections from hEN101-treated monkeys contained a higher fraction of both large and medium-sized IL-1β positive cell bodies than naïve sections, suggesting stress-induced inflammatory response (FIG. 4A, p=0.051 and 0.034 respectively, Wilcoxon test). Lower fractions of IL-1β labeled cell bodies were shown in sections from 500 µg/kg/day hEN101 i.v. as compared to 150 µg/kg/day p.o. treated monkeys (FIG. 4A, p=0.067 for both size groups, Wilcoxon test). Association analysis demonstrated a putative correlation between neuronal AChE-R and IL-1β levels in medium-sized, but not large cells (FIG. 4B and data not shown). IL-6 labeling as well was suppressed significantly following i.v. administration of 500 µg/kg hEN101 (FIG. 4C, p=0.03 and 0.015 for medium and large neurons, respectively) as compared to 500 µg/kg-p.o.-treated monkeys.

**Example 6**

Endotoxin Induces Impairments in AChE-R Activity and Integrity

[0262] Endotoxin administration produced a time-dependent decrease in plasma AChE activity, measured by quantifying the rate of ATrh hydrolysis in the presence of the butyrylcholinesterase (BChE) inhibitor iso-OMPA. This reduction displayed a significant treatment-by-time interaction (FIG. 5A) F(2,16)=3.94, p=0.04. Saline administration (placebo) caused no change in AChE activity, excluding the possibilities that it was induced by the injection stress or by circadian influences. The decline in hydrolytic activity could potentially reflect losses in the AChE protein. To test this possibility, electrophoretically separated plasma proteins were immune-reacted with antibodies selective for the C-terminal peptide unique to AChE-R [Sternfeld et al., (2000) id ibid.]. These antibodies labeled a 66 kd protein, likely to be full-length AChE-R, as well as a shorter peptide with an apparent size of 6.5 kDa. A parallel labeling pattern in the serum of stressed mice [Grisaru et al. (2001) id ibid.] raised the suggestion that this was an immunopositive C-terminus cleavage product of AChE-R. Endotoxin administration induced a slight, yet persistent, increase in the AChE-R cleavage product (FIG. 5B, 5C). This increase did not reach statistical significance F(1,8)=2.32, p=0.16, for treatment effect (FIG. 5C). However, at 9 hr post-treatment, the endotoxin-induced decrease in AChE activity was significantly correlated with endotoxin-induced increase in AChE-R cleavage (r=-0.65) (FIG. 5D).

**Example 7**

MALDI-TOF-MS Analysis of AChE-R Cleavage Product

[0263] To further characterize the AChE-R cleavage product, larger plasma samples (180 µg/mlane) were resolved by electrophoresis. Protein bands that co-migrated with the bands labeled with anti AChE-R antibodies were cut out of the gel and subjected to MALDI-TOF-MS analyses. The elution product of the larger band was identified as being mainly composed of serum albumin (molecular weight, 69,567), compatible with the assumption that AChE-R is only a minor component in this size fraction of human serum proteins. The shorter peptide eluted from the excised band, however, revealed a single peak with a molecular mass of 3613-3615. FIG. 6 demonstrates the MALDI-TOF-MS profile of this eluted peptide. Peptide property calculations positioned the presumed proteolytic cleavage site 36 residues from the C-terminus of AChE-R, with a calculated mass of 3614. Under these assumptions, cleavage could occur between asparagine and arginine residues upstream to the AChE-R diversion site (FIG. 6).

[0264] Parallel size peptides were observed in gel-eluted products from several individuals, demonstrating consistent cleavage processes. LysC proteolysis failed to further shorten this peptide. Edman degradation was unsuccessful, perhaps due to N-terminal blockade, and further experiments were prevented because of lack of material. The mass spectrometry approach thus pointed, although inconclusively, at an AChE-R cleavage site in human plasma under endotoxin stress near the C-terminal splice site that marks the deviation between human AChE splice isoforms.

**Example 8**

Vascular Endothelial Cells Produce AChE-R

[0265] In search for the potential cell type origin of plasma AChE-R, the inventors performed fluorescent in situ hybridization (FISH) and immunohistochemistry on human tissues from patients with or without inflammatory diseases (e.g. kidney vasculitis). Vascular endothelial cells displayed labeling with both AChE-R cRNA and anti AChE-R antibodies (FIG. 7A, 7B). Quantification of signal intensities revealed considerable similarities between AChE-R mRNA and AChE-R protein levels in patients with or without inflammatory vasculitis, so that tissues with less pronounced mRNA labeling also displayed fainter protein labeling (FIG. 7C). This pointed at vascular endothelial cells, which also harbor non-neuronal nicotinic acetylcholine receptors [Heesch et al., (2002) J. Clin. Invest. 110:527-36] as a probable site of continuous plasma AChE-R production.

**Example 9**

AChE-R Cleavage is Associated with Cytokines Secretion

[0266] Endotoxin induced a transient, significant increase in the plasma levels of cortisol, TNF-α and IL-6 (FIG. 8A-8C), although at the employed dose it does not produce any significant effects on the subjective rating of physical or behavioral sickness symptoms [Reichenberg (2001) id ibid.]. The selective increase in peripheral cytokine levels in the absence of subjective CNS effects on cognitive or
intellectual function, suggested that changes in memory functions under these conditions would reflect objective endotoxin-induced alterations. Cortisol levels increased during the first and second testing periods, TNF-α and IL-6 peaked during the first testing period and decreased thereafter and rectal temperature (not shown) peaked during the second period. These time-dependent effects were reflected by significant treatment-by-time interactions [F(2,16) = 41.2, 10.6, 10.5, 3.2, respectively, all p<0.05, by H-F].

[0267] At each testing period, correlation analysis enabled the comparison between the biochemical and functional responses of tested individuals. Thus, endotoxin-induced AChE-R cleavage (computed as the change in a certain individual from the endotoxin to the placebo condition) was significantly (p=0.05) and positively correlated with the secretion of cortisol, during the last testing period (r=0.70) (FIG. 8A). AChE-R cleavage was significantly (p=0.01) and negatively correlated with the secretion of TNF-α and IL-6 during the first (r=-0.72 and -0.66, respectively) (FIG. 8B, 8C), but not later testing periods.

Example 10
AChE-R Cleavage is Associated with Endotoxin-Induced Impairments in Declarative Memory

[0268] Endotoxin administration decreased the performance in tests of declarative memory during all testing periods. This was reflected by decreased immediate recall of story items [F(1,8)=6.5, p=0.03] (FIG. 9A) and reduced delayed story recall [F(1,8)=3.5, p=0.09] (data not shown). Endotoxin-induced decrease in immediate and delayed recall of story items was significantly (p<0.05) and negatively associated with TNF-α and IL-6 secretion (r=-0.59 to -0.67) during the first, but not during other testing periods (data not shown), suggesting the potential involvement of additional mechanisms(s) in endotoxin-induced impairments in declarative memory. At the last testing period, the endotoxin-induced decrease in immediate recall of story items was significantly (p=0.05) and negatively (r=-0.63) associated with AChE-R cleavage (FIG. 9B), indicating that the consequent increase in ACh levels, perhaps in conjunction with continuously suppressed cytokine production, interferes with declarative memory. This notion was supported by the positive (r=0.68) association of declarative memory impairments with the decrease in AChE activity during the last testing period (FIG. 9C), when cytokine levels already receded, but not during earlier testing periods.

Example 11
AChE-R Cleavage Association with Improved Working Memory

[0269] Endotoxin administration induced a significant improvement in working memory performance, reflected by an increased score in the digit span backward test during all testing periods [F(1,8)=12.3, p=0.008] (FIG. 10A). No significant changes in the digit span forward test (assessing memory span) or on the attention test (Ruff &/r7 cancellation test) were evident (data not shown), emphasizing the selectivity of the observed differences.

[0270] The endotoxin-induced improvement in working memory performance showed no significant association with the secretion of TNF-α, IL-6 or cortisol, yet was negatively associated with AChE-R cleavage. Association was significant (p<0.05) during the second and third testing periods (r=-0.84 and -0.64, respectively) (FIG. 10B and data not shown). Thus, subjects with a greater endotoxin-induced elevation in AChE-R cleavage (and, presumably, larger increases in ACh levels) showed both lower endotoxin-induced improvement in working memory functioning, and greater endotoxin-induced impairment in declarative memory.

Example 12
AChE-S Transgenic Mice Display Elevated Body Temperature

[0271] Fever is one of the consequences of higher levels of circulating pro-inflammatory cytokines. In order to verify whether the constitutive expression of human synaptic AChE (hAChE-S) [Beeri et al. (1995) id ibid.] and the consequent over-expression of murine AChE-R [Cohen et al. (2002) id ibid.] influenced the release of pro-inflammatory cytokines in the animal, the inventors measured body temperature. Five transgenic FVB/N hAChE-S and mAChE-R overexpressing females, 3-5 months old, had their temperature measured between 5 and 55 minutes after anesthesia, which was administered in order to induce a change in body temperature. As shown in the graph (FIG. 12A–B), body temperature decreased with post-treatment time. Interestingly, the average body temperature of the transgenic mice was always 2° C. higher than in the control mice. This suggests that their inherited cholinergic imbalance impaired their control over body temperature. These finding are compatible with the inventors’ previous report of impaired hypothermic response of these transgenic mice to the administration of paraoxon [Beeri et al., (1995) id ibid.].

Example 13
Effects of Tacrine on LPS-Induced IL-1 Secretion in the Hippocampus and IL-1 and TNF-α Secretion in the Serum

[0272] Male C57 mice were injected (i.p.) with either saline or tacrine (1.5 mg/kg), immediately followed by an injection of either saline or LPS (1.0 mg/kg) (n=5 animals per group). Two hours later, mice were deeply anesthetized with 24 µg Nembutal per mouse; blood was taken by heart puncture and the hippocampus was excised and placed in tubes containing 500 µl of RPMI+100 KIU aprotinin. The levels of IL-1β in the hippocampus (FIG. 13A) and IL-1β (FIG. 13B) and TNF-α (FIG. 13C) in the serum were assessed with commercial ELISA kits (R&D Systems). LPS induced a significant increase in the hippocampal and serum IL-1β, which was significantly attenuated in tacrine-treated mice. In contrast, tacrine produced a small and non-significant attenuation of LPS-induced TNF-α secretion in the serum.

Example 14
Effects of Rivastigmine on LPS-Induced IL-1 Secretion in the Hippocampus and IL-1 and TNF-α Secretion in the Serum

[0273] Male C57 mice were injected (i.p.) with either saline or one of three doses of rivastigmine (0.5, 1.5 and 3.0
Immediately followed by an injection of either saline or LPS (1.0 mg/kg) (n=5 animals per group). Two hours later, mice were deeply anesthetized with 24 µg Nembutal per mouse, blood was taken by heart puncture and the hippocampus was excised and placed in tubes containing 500 µl of RPMI1+100 KIU aprotinin. The levels of IL-1β, and TNF-α were assessed with commercial ELISA kits (R&D Systems). LPS induced a significant increase in the hippocampal IL-1β, which was significantly attenuated only by the high dose of rivastigmine (FIG. 14A). LPS-induced IL-1β secretion within the blood was dose-dependently suppressed by the 1.5 and 3.0 mg/kg doses of rivastigmine (FIG. 14B). LPS-induced TNF-α secretion in the blood was not affected by rivastigmine treatment, even at a high dose (FIG. 14C).

Example 15
Cytokines as Mediators of Emotional and Cognitive Effects of Stress Caused by Surgery

Several lines of evidence indicate that stress influences a variety of cognitive functions, including memory. In particular, exposure to stress was found to impair declarative memory, while leaving procedural memory intact. It is also well known that stress influences many immune functions, including the production and secretion of cytokines. Following exposure to various stressors, there is an increase in peripheral IL-6, as well as IL-1β and TNF-α, accompanied by decrease in IL-2, in both humans and experimental animals.

The study was designed to examine the role of cytokines in mediating the affective and cognitive effects of stress. Two types of stressful situations were investigated in the same subjects: Psychological stress—while waiting for a surgery (i.e., in the morning of the surgery day), and surgical stress—in the day after surgery.

Twenty generally healthy volunteers were administered with a comprehensive neuropsychological test battery, assessing emotional and cognitive parameters, before and after a minor surgery (Laparoscopic Cholecystectomy or Hernia). Each subject was tested in three occasions: (a) Several days before surgery (baseline); (b) by the morning of the surgery day=1, (c) A day after surgery=2. Blood samples were collected in each session, and serum levels of cytokines (IL-1β, IL-6) were measured. Fifteen control subjects went through the same procedure.

In the morning of the surgery day, there was a significant increase in the levels of both anxiety (STAI) (FIG. 15A) and depression (DACT). (FIG. 15B) (F(2,82)=3.871, p<0.025 and F(2,82)=11.189, p<0.0001, respectively). No change was found in the levels of fatigue and pain (FIGS. 15C and 15D, respectively). In the morning following surgery there was further increase in depression, but not in anxiety, alongside a significant increase in pain and fatigue (F(2,80)=24.588, p<0.0001 and F(2,80)=10.148, p<0.0001, respectively).

With regards to the cognitive parameters (FIG. 15E-15H), in the morning of the surgery day test showed a significant decline in performance of the word list recall task (IVL2) (F(2,70)=4.120, p<0.021). In the morning following surgery, an additional decline was found in the word list recall as well as in the performance of a visual memory task involving a complex figure reconstruction (MCC) (F(2,70)=3.973, p<0.023).

For each parameter (psychological performance, cytokine level, etc) differences were computed between each stressful situation (t1, t2) and baseline (t0). Pearson correlations were computed between cytokines levels and psychological variables (FIG. 16A-C).

In the morning of the surgery day (t1), there was a significant correlation between increased levels of IL-1β and the elevation in depressed mood (r=0.525) (FIG. 16B).

In the morning following the surgery (t2), there were significant correlations between increased IL-1 levels and impaired immediate and delayed Logical memory (story recall test) (r=-0.627 and -0.532, respectively). Significant correlations were obtained between increased IL-6 levels and improved delayed recall in the Word List Recall (IVL2) test (r=0.386, as well as improved immediate and delayed Complex Figure recall test (MCQ) (r=0.502 and 0.590, respectively). There was a significant increase in IL-6 (F(2, 35)=29.114, p<0.0001) (FIG. 16C).

Example 16
Selective Elimination of AChE-R mRNA in the Brain of EN301-Treated Mice
Experimental Procedure:

3 month old FVB/N female mice were injected intra-peritoneally daily with 500 µg/kg of EN301 (n=7) or with vehicle (PBS, n=6). EN301 corresponds to mEn101, defined herein as Seq 1D NO-2. This antisense oligonucleotide is targeted to a sequence within exon 2 of mouse AChE exon 2 sequence. EN301 was produced by Microsynth, Switzerland, at relatively large quantities for animal tests. The treatment persisted for 3 consecutive days, and the mice were sacrificed on day 4. Brain was collected, flash frozen in liquid nitrogen and stored at -70°C.

Total RNA was extracted from the brain and RT-PCR reaction was conducted using primers targeting the common sequence in Exon 2 of murine AChE cDNA or the unique sequence in Exon 6, specific to the AChE-S variant. 5 µl samples were removed from the 50 µl PCR reaction mixture at cycles 25, 31 and 35. Samples were run on a 1.5% Agarose gel. The results of the PCR specific for the exon 2 sequence, after 31 cycles, are shown in FIG. 17A. Photographs were saved and fluorescence quantified using the PhotoShop software, and the results expressed in histograms (FIGS. 17B-17C).

Results:

The goal of the present experiment was to test for reduction in AChE gene expression under EN301 treatment, while ensuring that AChE-S mRNA levels are maintained reflecting sustained cholinergic neurotransmission.

Normalized to RNA quantities, EN301-treated brains showed a significant 25% reduction (p=0.01, Student’s T-Test) in the common transcript levels (FIG. 17B), whereas the S variant showed a non-significant 17% increase (FIG. 17C), reflecting a relatively larger fraction of AchE-S mRNA out of the total content of mRNA as compared with the untreated brain.
The ratio between AChE-S:common (S/Com) transcripts showed that in the EN301-treated brain, the S/Com ratio is significantly increased (from 0.6 to 0.98). RT-PCR data cannot be used as such for comparing the absolute quantities of the analyzed transcripts, because different primer pairs may function with different efficacies. However, these results are significant and consistent with the finding that AChE-S mRNA was upregulated in the EN301-treated brains and that the relative concentration of AChE-S mRNA was increased, albeit insignificantly, under treatment. This supports the notion that the agent affects brain gene expression as well.

The present results lead to the conclusion that EN301 treatment causes selective destruction of AChE-R mRNA in the EN301 treated brains while maintaining essentially unmodified AChE-S levels. Note that to exert such an effect, EN301 does not necessarily have to cross the blood-brain barrier. Rather, by reducing the levels of peripheral AChE, it would increase acetylcholine levels, suppressing the production by macrophages of pro-inflammatory cytokines e.g. IL-1 [Wang H. et al. (2003) Nature 421, 384-8]. Because IL-1 promotes AChE gene expression [Li et al. (2000) J. Neurosci. 20, 149-155], and since the peripheral pro-inflammatory cytokines are known to affect the brain [Pick et al. (2004) Annals NY Acad. Sci. 1018, 85-98], such an effect will eventually reduce AChE-R levels in the brain as well.

Example 17

Animal Model for Guillain-Barré Syndrome and Inflammation-Associated Neuropathy

Intra-neural injection into a rat peripheral nerve is often used to study Guillain-Barré Syndrome GBS, testing the pathogenesis of the disease following nerve sheath impairment, i.e., examining the effect of intra-neural injection of reactive soluble factors, and not the nerve sheath impairment per se. Indeed, serum obtained from GBS patients was reported to cause demyelination and conduction blocks [Harrison B. et al., (1984) Ann. Neurol. 15: 163-170; Saida T. et al. (1982) Ann. Neurol. 11: 69-75], which are not elicited by intra-neural injection of anti-GM1 IgG or IgM [Harvey G. et al. (1995) Muscle Nerve 18: 388-394].

The inventors previously employed an animal model for GBS to test the effect of systemic exposure to Cj-LPS on the sciatic nerve [Ihergane (2003) id ibid.] Following pre-sensitization with the immune responses activator—keyhole limpet hemocyanin (KLH), rats were systemically exposed to Cj-O:19 LPS via intraperitoneal (i.p.) injection. Parallel to LPS exposure, minor focal sciatic nerve trauma was applied by intra-neural (i.n.) injection of saline (FIG. 18A).

Compound muscle action potential (CMAP) stimulated proximal to the neural injection site appeared lower than distally stimulated CMAP (referred as reduced proximal to distal ratio; PDR, see FIG. 18B,18C). This indicates nerve conduction blocks (when PDR<0.5) which developed in rats that received an i.n. injection of saline concomitantly with the systemic Cj-LPS exposure. Conduction blocks appeared 1-3 days after the LPS exposure and spontaneously resolved after 8 days. Conduction blocks did not develop in rats that were systemically exposed to Cj-LPS without an intraneural injection, and neither in rats which were injected intraneurally directly with the Cj-LPS itself. Conduction blocks developed in 3 out of 10 intraneurally injected rats which were not exposed to Cj-LPS and in none of the animals which were intraneurally injected 8 days after Cj-LPS exposure. The differences between the test and control groups were statistically significant (P<0.01). Morphological analysis of the injected nerves revealed no morphological abnormalities (i.e. demyelination, axonal degeneration or inflammatory changes) on days 3 and 9 following i.n. injection, in either group. The fact that direct Cj-LPS i.n. injection did not result in conduction abnormality, suggests a non-direct mechanism, in which the Cj-LPS stimulates systemic production of a factor that causes conduction block in the peripheral nerves that it penetrates. Furthermore, similar results were observed in rats treated with E. Coli LPS. All the animals which were concomitantly treated by i.p. E. Coli LPS and i.n. saline, developed conduction blocks on one day following injection (average PDR=0.47, S.D. 0.06), which resolved in the following 2 days. This indicates that a systemic reaction common to both gram negative bacilli LPS induces a neural reaction if soluble factors penetrate through the nerve sheath.

To test this hypothesis, splenocytes from KLH pre-sensitized rats were exposed to Cj-LPS in a cell suspension for 48 hours. The medium of splenocytes which reacted with LPS in vitro (reactive splenocyte medium) was then injected intraneurally to the sciatic nerve of rats. This reactive medium was cell-free and devoid of IgG or IgM, and did not elicit any electrophysiological effect within 10 minutes following injection, indicating that it did not contain neuroinhibitory or toxic substances reminiscent of curare or tetrodotoxin which typically block ion channels within minutes. Nevertheless, one to 4 days following i.n. injection, CMAP stimulated proximal to the injection site was reduced in more than 70% of nerves, indicating a conduction block. Normal splenocyte medium (not reacted with LPS) induced a conduction block in only 6.2% of nerves which was significantly different (p<0.01). Conduction block duration was 1.44±1.02 days with resolution in 70% of the nerves. In this case as well, morphological analysis demonstrated no demyelination, axonal degeneration or inflammatory abnormalities, indicating that the electrophysiological pathology was not due to gross neural deformity, or myelin sheath and axonal degeneration. These results strengthen the hypothesis that an immune reaction to Cj-LPS similar to E. Coli LPS, produces a soluble factor which induces functional conduction abnormalities within penetrated nerves while the neural structures are yet preserved.

Example 18

Treatment with EN101 Inhibits Conduction Blocks

The inventors tested the participation of AChE-R in the sequence of events that follow exposure to LPS and lead to conduction abnormalities in the GBS model described above. Indeed, systemic (i.p.) treatment with EN101 (0.5 mg/kg) prevented formation of conduction blocks when applied with Cj-LPS parallel to i.n. saline injection. Treatment with EN101 significantly improved PDR (proximal to
distal amplitude ratio, p<0.01), which became similar to non-i.n. injected controls (p=0.45; FIG. 19A). Furthermore, addition of EN101 (20 pmole) to i.n. injections of C3-LPS reactive splenocyte medium prevented the appearance of nerve conduction block and reduction in PDR compared to control group injected with LPS-reacted splenocyte medium alone (p<0.05; FIG. 19B). Additionally, to simulate macrophage intra-neural invasion as detected in GBS, bone marrow-derived cultured macrophages were intra-neurally injected. These cells induced conduction blocks which initiated 1 day following injection, with resolution after 7±1 days. Concomitant i.n. EN101 injection abolished the appearance of conduction blocks or reduction in PDR (p<0.01; FIG. 19C). Furthermore, addition of EN101 by i.p. injection at the time of conduction block in non-EN101 treated injected nerves on day 1, increased PDR on the following day compared to animals that did not receive i.p. EN101 (t-test p<0.05). The direct causal effect of AChE-R on nerve conduction was examined by i.n. injection of synthetic ARP (1.4 nmole) to the sciatic nerve of adult naïve rats. This treatment produced a transient nerve conduction block, which initiated 24 hrs following injection and lasted 48 hrs (FIG. 19D). Injection of AChE-Synaptic peptide (ASP; 1.4 nmole) as a negative control did not produce a conduction block, indicating the specificity of ARP for conduction blockade (p<0.05).

[0293] In a recent study, mild stress was shown to induce AChE-R in the hippocampus, and to interact intraneuronally with a scaffold protein RACK1 and through it, with its target, protein kinase CβII (PKCβII), in a manner suppressible by antisense prevention of AChE-R accumulation [Birikh (2003) id. ibid.; Njiholt, I. et al., (2004) Molecular Psychiatry 9: 174-183]. In agreement with this, the inventors identified that LPS-reacted splenocyte medium i.n. injection increased PKCβII levels in sciatic nerve by immunoblot analysis, which was suppressed by EN101 treatment (FIG. 20). These results further support the hypothesis that AChE-R plays a key role in induction of functional nerve conduction blocks following immune activation by LPS exposure or i.n. invasion of macrophages, as evidenced in GBS, and strongly suggest that ARP is the active modulator in these processes. Nevertheless, AChE-R induction is not restricted to reaction to the Campylobacter type of LPS. Furthermore, the formation of nerve conduction block by direct i.n. injection of ARP indicates that AChE-R and ARP may affect nerve conduction in response to various inflammatory responses, where nerve sheath/blood nerve barrier is injured or disrupted, as exemplified in GBS. Hence, EN101 treatment to treat nerve conduction pathology is applicable for conditions that similarly induce AChE-R when concurrent nerve sheath disruption is present.

SEQUENCE LISTING

SEQ ID NO 1 LENGTH 20 TYPE DNA ORGANISM: Artificial sequence FEATURE AChE

SEQ ID NO 1 Sequences:
ctgccacgtt ctcttgaccc

SEQ ID NO 2 LENGTH 20 TYPE DNA ORGANISM: Artificial sequence FEATURE AChE

SEQ ID NO 2 Sequences:
ctgcacatatt ttcttgtacc

SEQ ID NO 3 LENGTH 20 TYPE DNA ORGANISM: Artificial sequence FEATURE AChE

SEQ ID NO 3 Sequences:
ctgcacatatt ttcttgaccc

OTHER INFORMATION: Antisense oligonucleotide hEN101 targets human AChE

OTHER INFORMATION: Antisense oligonucleotide mEN101 (EN301), targets mouse AChE

OTHER INFORMATION: Antisense oligonucleotide rEN101, targets rat AChE
1. A method of treatment of a condition triggering an inflammatory response in a mammalian subject, comprising administering to the subject a therapeutically effective amount of an inhibitor of AChE expression or a pharmaceutical composition comprising the same.

2. A method for the treatment and/or prevention of inflammation in the joints, central nervous system, gastrointestinal tract, endocardium, pericardium, lung, eyes, skin and urogenital system of a mammalian subject, comprising administering to the subject a therapeutically effective amount of an inhibitor of AChE expression or a pharmaceutical composition comprising the same.

3. A method for suppressing the release of a pro-inflammatory cytokine, comprising administering a therapeutically effective amount of an inhibitor of AChE expression or a pharmaceutical composition comprising the same, to a subject.

4. A method for treating fever, comprising administering a therapeutically effective amount of an inhibitor of AChE expression or a pharmaceutical composition comprising the same, to a subject.

5. A method for the treatment of an inflammation-associated neuropathy, comprising administering a therapeutically effective amount of an inhibitor of AChE expressions or a pharmaceutical composition comprising the same, to a subject.

6. The method of claim 5, wherein said inflammation-associated neuropathy is Guillain-Barré Syndrome.

7. The method of claim 1, wherein said inhibitor of AChE expression is any one of an AChE-specific ribozyme, an RNA sequence used for RNA interference of the AChE gene, or an antisense oligonucleotide directed against AChE.
8. The method of claim 1, wherein said inhibitor of AChE expression is a nuclease resistant antisense nucleotide directed against AChE or a functional analog, derivative, or fragment thereof.

9. The method of claim 7, wherein said inhibitor of AChE expression is an antisense oligonucleotide directed against AChE, having the sequence as denoted by any one of SEQ ID No:1, SEQ ID No:2 or SEQ ID No:7 or a functional analog, derivative, or fragment thereof.

10. The method of claim 7, wherein said inhibitor of AChE expression is an antisense oligonucleotide directed against AChE, having the sequence as denoted by SEQ ID NO:1, or a functional analog, derivative, or fragment thereof.

11. The method of claim 3, wherein said pro-inflammatory cytokine is selected from the group consisting of IL-1β, TNFα, IL-6, IL-8, IL-12, and IL-18.

12. The method of claim 3, wherein said pro-inflammatory cytokine release is triggered by one of stress, bacterial infection, drugs, irradiation, exposure to AChE inhibitors, stroke, auto-immune diseases, multiple chemical sensitivity, and any cumulative age-dependent damages.

13. The method of claim 1, wherein said mammalian subject is a human, and said inhibitor of AChE expression is an antisense oligonucleotide directed against AChE, as denoted by the sequence selected from SEQ ID NO:1 and SEQ. ID. NO:7, or a functional analog, derivative, or fragment thereof.

14. The method of claim 10, wherein said antisense oligonucleotide or composition comprising the same is for daily use by the subject, and said therapeutically effective amount is a dosage of active ingredient between about 0.001 μg/g and about 50 μg/g.

15. The method of claim 14, wherein said dosage of active ingredient is between about 0.01 and about 5.0 μg/g.

16. The method of claim 15, wherein said dosage of active ingredient is between about 0.15 and about 0.50 μg/g.

17. The method of claim 1, wherein said conditions are selected from any one of stress, bacterial infection, drugs, irradiation, exposure to AChE inhibitors, stroke, auto-immune diseases, multiple chemical sensitivity and any cumulative age-dependent damages.

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