tonomy of the patients in daily living. These results open promising perspectives for the treatment of other heredodegenerative secondary dystonias in children and adult patients.

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References


Acetylcholinesterase Inhibitors Reduce Brain and Blood Interleukin-1β Production

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Overproduction of interleukin-1 within the brain is associated with Alzheimer’s disease and other neurological conditions. We report that peripheral administration of the acetylcholinesterase inhibitors tacrine, rivastigmine, neostigmine, or EN101 (an antisense oligonucleotide directed at acetylcholinesterase messenger RNA) to mice significantly attenuated the production of interleukin-1β in the hippocampus and blood, concomitantly with the reduction in acetylcholinesterase activity. These findings demonstrate that cholineric enhancement produces central and peripheral antiinflammatory effects and suggest a novel therapeutic mechanism for acetylcholinesterase inhibitors.

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Interleukin-1 (IL-1) is a pleiotropic protein, produced mainly by cells of the macrophage/microcyte lineage, as well as by glia and neurons within the brain. IL-1 exerts important physiological effects on a number of different target cells involved in inflammatory and immune responses. However, excessive production and secretion of IL-1 causes highly detrimental effects in

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inflammatory and autoimmune diseases. Moreover, several lines of evidence point to a potential role for IL-1 in the pathophysiology of neurodegenerative diseases, including Alzheimer’s disease (AD).2

Several endogenous systems evolved to regulate the production and actions of IL-1, including the production and secretion of IL-1 receptor antagonist (IL-1ra) and other antiinflammatory cytokines, the IL-1 type II “decoy” receptor,3 and several hormones with antiinflammatory effects.4 Recent findings indicate that neural mechanisms also are involved in limiting inflammatory responses. In particular, it was found that acetylcholine (ACh) inhibits lipopolysaccharide (LPS)–induced production of proinflammatory cytokines, including IL-1, from macrophages5 and microglia.6

The levels of ACh are continuously regulated by the hydrolytic enzyme acetylcholinesterase (AChE), which rapidly degrades ACh both in the periphery and the brain. AChE inhibitors are potent cholinergic agonists7 and are widely accepted as anti-AD drugs.8 Therefore, we examined the effects of these drugs on the production of IL-1β within the hippocampus, one of the brain areas where structure and function are most affected in AD,9 as well as in the blood, after peripheral administration of LPS in mice. To test directly the causal effect of AChE levels, we used EN101, an antisense oligonucleotide capable of suppressing brain AChE levels after peripheral administration in mice.10

Materials and Methods

Mice

Male C57/BL6 mice aged 8 to 12 weeks (Harlan) were housed at 23±1°C on a 12-hour light/dark cycle (light on at 9:00 AM). Laboratory animal experiments were approved by the Hebrew University’s Committee for Animal Experimentation.

Materials

Tacrine was purchased from Tocris (Avonmouth, UK), and neostigmine was purchased from Sigma (Rehovst, Israel). Rivastigmine generously was provided as a gift from Dr M. Weinstock. Lyophilized 2’-O-methyl–protected (three 3’-nucleotides) oligonucleotides were injected intraperitoneally (500µg/kg). The EN101 antisense oligonucleotide is complementary to the coding sequence of the mouse AChE messenger RNA sequence (GenBank accession number S50879) common to all variants. The inverse sequence to EN101 served as a negative control.

Procedure

In four separate experiments, mice were injected intraperitoneally with an AChE inhibitor (tacrine, 1.5mg/kg; rivastigmine, 3mg/kg; neostigmine, 0.15mg/kg; or EN101, 500µg/kg) or with saline, followed by another intraperitoneal injection (24 and 1 hour later for EN101 and immediately for all other AChE inhibitors) of either LPS (1mg/kg) or saline. Mice were overdosed with a Nembutal injection (60mg/kg) 2 hours after the LPS injection; blood was drawn by cardiac puncture and centrifuged for 15 minutes (300g) at 4°C. Hippocampi were removed rapidly and placed in 200µl cold RPMI-1640 containing 0.5µl protease inhibitor cocktail (Sigma), homogenized, and centrifuged for 15 minutes (300g) at 4°C. IL-1β levels were determined using mouse IL-1β enzyme-linked immunosorbent assay kits (R & D Systems, Minneapolis, MN). Total protein was determined by the Bradford method. In another experiment, the same procedure was used to determine the effects of the four AChE inhibitors on AChE activity in the hippocampus and the blood, using the Ellman method.10

Statistical Analysis

Data (means ± standard error of the mean) were analyzed first by analyses of variance, and then by planned contrasts or post hoc comparisons (Tukey’s test, p < 0.05).

Results and Discussion

Mean basal level of IL-1β in the hippocampus was 6.3pg/mg protein. AChE inhibitors had no effect on basal levels of IL-1β in the hippocampus (F(4,22) = 0.68; p > 0.05; Fig 1). Mean basal level of IL-1β in the blood was 6.6pg/ml. Overall, administration of AChE inhibitors was associated with a reduction of basal levels of IL-1β in the blood (F(4,22) = 10.9; p < 0.001). Post hoc analysis indicated significant reductions of basal IL-1β only after tacrine and rivastigmine administration, but not after neostigmine or EN101 treatment (see Fig 1).

In all experiments, LPS produced a significant increase in IL-1β production, both in the hippocampus (mean, 24.1pg/mg protein) and in the blood (mean, 228pg/ml) (p < 0.01).

In the hippocampus, LPS-induced production of IL-1β was reversed by AChE inhibitors (F(4,24) = 11.3; p < 0.001). Post hoc analysis indicated significant reduction of IL-1β levels by all the inhibitors. Similarly, in the blood, LPS-induced production of IL-1β was significantly attenuated by AChE inhibitors (F(4,24) = 8.6; p < 0.001). Post hoc analysis indicated significant reduction of IL-1β levels by tacrine, rivastigmine, and EN101, and a tendency toward significant effect of neostigmine (p = 0.07; see Fig 1).

Overall, LPS produced a small decrease in AChE activity, both in the hippocampus and in the blood (7 and 4%, respectively), which did not reach statistical significance (p > 0.1; Fig 2). Regardless of LPS administration, AChE activity levels in the hippocampus were significantly attenuated by tacrine, rivastigmine, and EN101 (F(4,64) = 6.3; p < 0.001), but not by neostigmine. In the blood, AChE activity was significantly reduced by all AChE inhibitors (F(4,60) = 4.3; p < 0.01; see Fig 2).

Reduction in AChE activity in the blood was larger after treatment with tacrine and rivastigmine (52 and
41%, respectively) compared with neostigmine and EN101 (28 and 21%, respectively). Remarkably, the reduction in IL-1β levels in the blood followed the same pattern. Tacrine and rivastigmine were more effective in attenuating IL-1β production in the blood (74 and 79%, respectively) compared with neostigmine and EN101 (41 and 47%, respectively).

The results indicate that systemic administration of LPS produces a marked increase in IL-1β levels, both in the hippocampus and in the periphery, corroborating many previous reports that LPS and other peripheral immune challenges induce the production of IL-1β in the brain, in general, and the hippocampus, in particular. Furthermore, we report that concomitantly with their hydrolytic effect, peripheral administration of AChE inhibitors almost completely blocked the LPS-induced increase in IL-1β production in the hippocampus and attenuated LPS-induced secretion of

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**Hippocampus**

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**Serum**

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Fig 1. Effect of acetylcholinesterase (AChE) inhibitors on lipopolysaccharide (LPS)-induced interleukin (IL)-1β production in the hippocampus (left four panels) and the blood (right four panels). Male C57Bl mice were injected intraperitoneally with either saline or one of four AChE inhibitor (tacrine, rivastigmine, neostigmine, or EN101), followed by a second injection of either saline or LPS (1mg/kg). IL-1β production 2 hours after LPS injection was measured by enzyme-linked immunosorbent assay. Data represent the mean (± standard error of the mean) of five to nine mice per group.
IL-1β in the blood. These findings demonstrate, for the first time, that AChE inhibitors have an anti-inflammatory property, both in the brain and in the periphery.

Recent studies show that in the periphery, cholinergic neurons of the efferent vagus nerve inhibit acute inflammation, providing a rapid, localized, and adaptive antiinflammatory reflex system.5 Specifically, ACh, which is secreted by the vagal efferents, inhibits LPS-induced secretion of tumor necrosis factor-α and IL-1β by macrophages, as well as by microglia, through the α7 unit of the nicotinic receptor that is expressed by these cells.5,6 The findings of this study demonstrate that cholinergic stimulation also can affect the in vivo production of a proinflammatory cytokine in the brain, suggesting that increased levels of ACh within the brain directly inhibit IL-1β production by hippocampal microglia.

In addition to a direct effect of AChE inhibitors in the brain, it also is possible that the peripheral ACh increase produced by these drugs suppressed the production of proinflammatory cytokines by macrophages, which, in turn, decreased the inflammatory signal to the brain, resulting in attenuated production of brain IL-1β. Our findings with the AChE inhibitor neostigmine, which does not cross the blood–brain barrier, provide strong support for this hypothesis. As shown here, neostigmine had no effect on the activity of AChE within the brain, but it completely blocked the production of IL-1β within the hippocampus, suggesting that the peripheral effect of AChE inhibitors contributes to the prevention of IL-1β overproduction within the brain.

A possible shortcoming of this interpretation is that both neostigmine and EN101, which were less effective in attenuating IL-1β levels in the periphery, still completely blocked IL-1β production in the hippocampus. One possible explanation for this discrepancy is that to increase the levels of IL-1β within the brain, LPS-induced production of IL-1β in the periphery must exceed a fairly high threshold.14 Accordingly, it is possible that a relatively small reduction of peripheral IL-1β would be translated into a greater attenuation of brain IL-1β production.

In this study, we induced acute endotoxemia, which is an established model of both central and peripheral production of IL-1. The finding that AChE inhibitors modulated IL-1β production in this model, together with findings of long-term inhibition of AChE activity by chronic treatment with AChE inhibitors,15 should prompt research on more chronic inflammatory conditions, including transgenic models of AD.

The findings that AChE inhibitors can reduce the production of IL-1β may have important clinical implications. Excessive production and secretion of IL-1 have been shown to produce highly detrimental effects in many inflammatory conditions.4 Currently, the clinical literature on the possible use of cholinergic agents as antiinflammatory drugs is limited.16,17 Our data suggest that AChE inhibitors might be valuable in the treatment of inflammatory diseases.

AChE inhibitors are considered beneficial and frequently are prescribed for patients with AD.8 The involvement of IL-1 in AD is suggested by the increased expression of IL-1 in the brains of patients with AD, the increased risk for development of AD in people with specific IL-1 polymorphisms, and the induction of amyloid precursor protein expression, tau hyperphosphorylation, and AChE production by IL-12,18 Our current findings, together with recent findings that AChE inhibitors downregulate IL-1β, IL-6, and tumor necrosis factor-α in peripheral blood mononu-
clear cells of patients with AD,19,20 implicates the inhibition of brain IL-1 as a novel mechanism of action for the beneficial effect of AChE inhibitors in AD.

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References