Eye drop NGF administration promotes the recovery of chemically injured cholinergic neurons of adult mouse forebrain

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Abstract
We have recently shown that conjunctivally applied nerve growth factor (NGF) in rats can reach the retina, the optic nerve and the CNS. In the present study, we investigated whether NGF application as collyrium can promote the recovery of chemically injured basal forebrain cholinergic neurons. NGF was administered on the eye of adult male mice previously treated i.c.v. with ibotenic acid to impair cholinergic pathways. Mice were tested in the passive avoidance test, and after 2 weeks of NGF administration were killed and the brains used for structural, biochemical and molecular analyses. The results showed that application of NGF on the eye surface protected choline acetyl transferase levels. These findings strengthen the hypothesis that application of NGF on the eye can represent an alternative delivery route to promote the recovery of brain cells during degeneration, including neurons involved in learning and memory activities.

Introduction
Nerve growth factor (NGF) is a neurotrophin that is able to promote nerve cell survival and neurite outgrowth in a number of neurons of the peripheral and CNS (Levi-Montalcini, 1987; Connor & Dragunow, 1998; Aloe et al., 2001), after binding and activating the low- and high-affinity receptors, p75 and TrkA, respectively (Barbacid, 1995; Ebendal, 1992; Liberini & Cuello, 1994; Sofroniew et al., 2001). Basal forebrain cholinergic neurons (BFCN) include cells of the medial septum that project to the hippocampus and of the nucleus basalis magnocellularis, which innervate the cortex. These neurons are known to undergo degeneration during brain ageing and memory loss-related disorders (Coyle et al., 1983; Fischer et al., 1991; Connor & Dragunow, 1998; Sofroniew et al., 2001). Other studies demonstrated that surgical transection or chemical injuries of these nerve fibres may cause loss of NGF receptors and reduce choline acetyl transferase (ChAT) activity (Hagg et al., 1989; Fischer et al., 1991; Liberini & Cuello, 1994). Results obtained on animal models showed that reduction of brain NGF synthesis can lead to neuronal plasticity impairment and memory deficits, while endocerebral administration of NGF can rescue degenerating BFCN (Hagg et al., 1989; Fischer et al., 1991). However, intracerebroventricular (i.c.v.) administration of NGF is a rather invasive approach, and the results can lead to debated interpretations. In recent years, other attempts to deliver NGF into the brain, such as to bind NGF to transferrin (Friden et al., 1993; Kordower et al., 1994), the use of biological mediators to promote NGF synthesis (Tirassa et al., 1999), gene therapy (Tuszynski et al., 2005) and nasal spray (Chen et al., 1998), have been made.

We have previously shown that radiolabelled 2.5S NGF administered as collyrium on the eye surface can reach the retina and optic nerve (Lambiase et al., 2005). More recently, we reported that NGF administered as eye drops can reach forebrain cholinergic neurons, suggesting that the mechanism through which NGF reaches brain neurons includes anatomical connections between the eye and the brain, or via nasal mucosa and naso-lacrimal duct (Ambati et al., 2000; Koevary et al., 2002; Koevary, 2003; Lambiase et al., 2007). To further characterize our previous findings, we have investigated the effect of NGF application on eye surface of chemically injured BFCN through i.c.v. administration of ibotenic acid (IBO) to cause loss of neurons (Aloe, 1987; Triaca & Aloe, 2005).

Materials and methods
Animals
For this study we used 90-day-old CD-1 male mice (n = 36) raised in our stabularium and kept under standard conditions (12 h light : 12 h dark cycle) with free access to water and food (Purina chow food). For the housing, care and experimental procedures, we followed the guidelines indicated and approved by CNR, Italian National Research Council and in conformity with the Intramural Committee and Institutional Guidelines in accordance with national and international laws (EEC council directive 86/609, OF L 358, 1, 12 December 1987). All efforts were taken to limit the number of experimental subjects.
Table 1 shows the experimental procedures used in the study.

Passive avoidance testing
Animals were tested for learning abilities in the passive avoidance box 1 week before the IBO injection to create a habituation profile, 1 week after the IBO injection and 3 days after the end of the NGF treatment on the eye surface. In the present experimental design we followed, with minor modification, methods previously described.