Ocular nerve growth factor administration counteracts the impairment of neural precursor cell viability and differentiation in the brain subventricular area of rats with streptozotocin-induced diabetes

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Abstract
The ocular administration of nerve growth factor (NGF) as eye drops (oNGF) has been shown to exert protective effects in forebrain-injured animal models, including adult diabetes induced by a single injection of streptozotocin (STZ) (60 mg/kg body weight). This type 1 diabetes model was used in this study to investigate whether oNGF might extend its actions on neuronal precursors localised in the subventricular zone (SVZ). NGF or saline was administrated as eye drops twice daily for 2 weeks in rats with STZ-induced diabetes and healthy control rats. The expression of mature and precursor NGF and the NGF receptors, tropomyosin-related kinase A and neurotrophin receptor p75, and the levels of DNA fragmentation were analysed by ELISA and western blotting. Incorporation of bromodeoxyuridine was used to trace newly formed cells. Nestin, polysialylated neuronal cell adhesion molecule (PSA-NCAM), doublecortin (DCX) and glial fibrillary acidic protein antibodies were used to identify the SVZ cells by confocal microscopy. It was found that oNGF counteracts the STZ-induced cell death and the alteration of mature/pro-NGF expression in the SVZ. It also affects the survival and differentiation of SVZ progenitors. In particular, oNGF counteracts the reduction in the number of cells expressing PSA-NCAM/DCX (neuroblast type A cells) and the related reductions in the number and distribution of nestin/DCX-positive cells (C-type cells), or glia-committed cells (type B cells), observed in the SVZ of diabetic rats. These findings show that oNGF treatment counteracts the effect of type 1 diabetes on neuronal precursors in the SVZ, and further support the neuroprotective and reparative role of oNGF in the brain.

Introduction
In mammals, including humans, the subventricular zone (SVZ) lining the forebrain lateral ventricles is the richest source of new cells in the brain (Alvarez-Buylla & Garcia-Verdugo, 2002), and maintains the ability to generate the three main cell types of the mature nervous system, such as neurons, astrocytes, and oligodendrocytes, in adult life (Doetsch et al., 1997; Alvarez-Buylla & Lim, 2004).

Neuronal damage acts as a potent stimulus for SVZ neuronal precursors by activating proliferation, migration and differentiation into neuron or glia phenotypes to promote neuronal and functional repair (Curtis et al., 2007). The response of the SVZ to damage is regulated by endogenous factors, including cytokines, growth factors, and neurotrophic factors, which can be released at the sites of injury in various pathological conditions, including neurodegenerative diseases, stroke, and diabetes (Guo et al., 2010; Christie & Turnley, 2013).

The neurotrophin nerve growth factor (NGF) is an endogenous factor that promotes the survival and differentiation of precursor neuronal cells throughout life, from development to aging, and it has been suggested as a potential therapeutic target to upregulate neurogenesis in the brain (Curtis et al., 2007; Bath & Lee, 2010).

Increased survival of SVZ cells is observable following induction of NGF synthesis in the brain in a model of psychosocial stress in aged mice (Fiore et al., 2003) and following environmental enrichment in adult rats (Ickes et al., 2000; Plane et al., 2008). Intracerebrally injected NGF also stimulates differentiation of SVZ neuronal precursor cells in aged mice (Ickes et al., 2000; Plane et al., 2008) and in animal models of neuroinflammatory brain diseases (Colza et al., 2003; Triaca et al., 2005), indicating that the administration of exogenous NGF can mimic the effect of the endogenous form on immature neuronal cells.

Similarly to what was observed following intracerebral administration, NGF has been proved to exert biological actions in the brain when applied as eye drops (oNGF) by activating c-fos expression in limbic structures (Colza et al., 2011) and in the SVZ (Tirassa, 2011) of healthy adult rats, and by stimulating the expression of