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BRAIN RESEARCH

Research Report

Dual effects of TNFα on nerve fiber formation from ventral mesencephalic organotypic tissue cultures

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ABSTRACT

Tumor necrosis factor alpha (TNFα) is toxic to dopamine neurons and increased levels of TNFα are observed in Parkinson's disease. Dopamine nerve fiber outgrowth in organotypic cultures of fetal ventral mesencephalon occurs in two waves. The early appearing nerve fibers are formed in the absence of astroglia, while migrating astrocytes guide the late appearing dopamine nerve fibers. TNFα (40 ng/ml) was added to the medium of organotypic ventral mesencephalic tissue cultures between days 4–7 and 11–14. The cultures were evaluated at days 7 or 19 to study the effects of TNFα on both types of nerve fiber formation. Tyrosine hydroxylase (TH)-immunohistochemistry demonstrated that the number of cultures showing non-glial-guided TH-positive outgrowth was reduced compared to controls, when TNFα was added at day 4. By contrast, the glial-guided TH-positive nerve fiber outgrowth and the astrocytic migration reached significantly longer distances by early TNFα treatment. Ki67-immunohistochemistry revealed that TNFα did not affect proliferation of astrocytes. Treatment with TNFα and antibodies against TNFα receptor 1 between days 4 and 7 revealed that the non-glial-guided TH-positive outgrowth reappeared. TNFα treatment between days 11 and 14 triggered neither the TH-positive glial-guided outgrowth, nor promoted the astrocytic migration to reach longer distances. The number of microglia was significantly increased after the late but not early TNFα treatment. In conclusion, TNFα is toxic for the non-glial dopaminergic nerve fiber outgrowth but stimulates the glial-guided outgrowth and the migration of astrocytes at an early time point. TNFα increased the number of microglia in VM tissue cultures after late but not after early treatment.

1. Introduction

In neurodegenerative diseases, such as in Parkinson's disease, neuroinflammation plays a critical role. During neuroinflammation as well as in Parkinson's disease, the levels of proinflammatory cytokines are elevated (Boka et al., 1994; Mogi et al., 1994; Sawada et al., 2006). Tumor necrosis factor alpha (TNFα) belongs to the family of proinflammatory cytokines. The levels of TNFα are elevated in Parkinson's disease, and TNFα is known to induce apoptotic cell death (Kolesnick and Golde, 1994; Mogi et al., 1994). TNFα is produced by activated microglia and the number of activated microglial cells is increased in the brains of parkinsonian patients (McGeer and McGeer, 1997). Microglia are already activated during the early

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phase of Parkinson's disease, and the presence of activated microglia is correlated with decrease in dopamine nerve fiber density (Ouchi et al., 2005).

It has been shown that dopamine neurons are vulnerable to TNFα in tissue culture (McGuire et al., 2001). Furthermore, it has been documented that the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is less effective in TNFα deficient mice (Ferger et al., 2004), suggesting that TNFα takes part in the degenerative process. However, TNFα can also protect injured dopamine neurons in tissue culture (Shinpo et al., 1990). This neuroprotective effect is further confirmed in an in vivo study where the presence of TNFα at an early, transient event after injury of the dopamine system is beneficial for dopamine cell survival and regeneration (Gemma et al., 2007). Thus, there are indications that TNFα exerts a dual effect.

TNFα exerts its effect via two receptors, TNF receptor 1 (TNFR1) and TNF receptor 2 (TNFR2) (Lewis et al., 1991; Tartaglia et al., 1993, 1991). These receptors are localized on neurons, including the nigral neurons, as well as on glial cells (Boka et al., 1994; Dziewulska and Mossakowski, 2003; McGuire et al., 2001). TNFR1 induces cell death via a cascade of death proteins while TNFR2 instead stimulates survival and migration. Moreover, double mutant mice for TNFR1 and TNFR2, but not single mutants for either one of the two receptors, are protected against mechanical or toxic insults to the dopamine neurons (Rousselet et al., 2002; Sriram et al., 2002; Wessig et al., 2005). Thus, although TNFR2 is supposed to exert neuroprotective properties, both receptors need to be blocked to prevent degenerative effects of TNFα.

Astrocytes and microglia are mobilized after injury, infection, or in neurodegenerative diseases. Specially, the loss of dopaminergic neurons in Parkinson's disease is associated with an increased glial reaction. Astrocytes respond particularly to proinflammatory cytokines and it is believed that cytokines participate in astrocyte activation (McNaught and Jenner, 2000). In organotypic slices cultures of fetal ventral mesencephalon (VM), dopamine neurons form their nerve fibers either in the absence of astroglial cell bodies or onto a monolayer of astrocytes (Johansson and Strömberg, 2002). The two sequences of outgrowth are temporally separated, and the first formed nerve fibers appear prior to proliferation and migration of astroglia. When the astrocytes proliferate, they also migrate away from the tissue slice and form a monolayer to finally surround the tissue slice. As the astrocytes start to migrate, the initially formed nerve fibers are retracted and the secondary formed nerve fibers grow onto the astrocytes. Both types of outgrowth are often visible in the same culture over

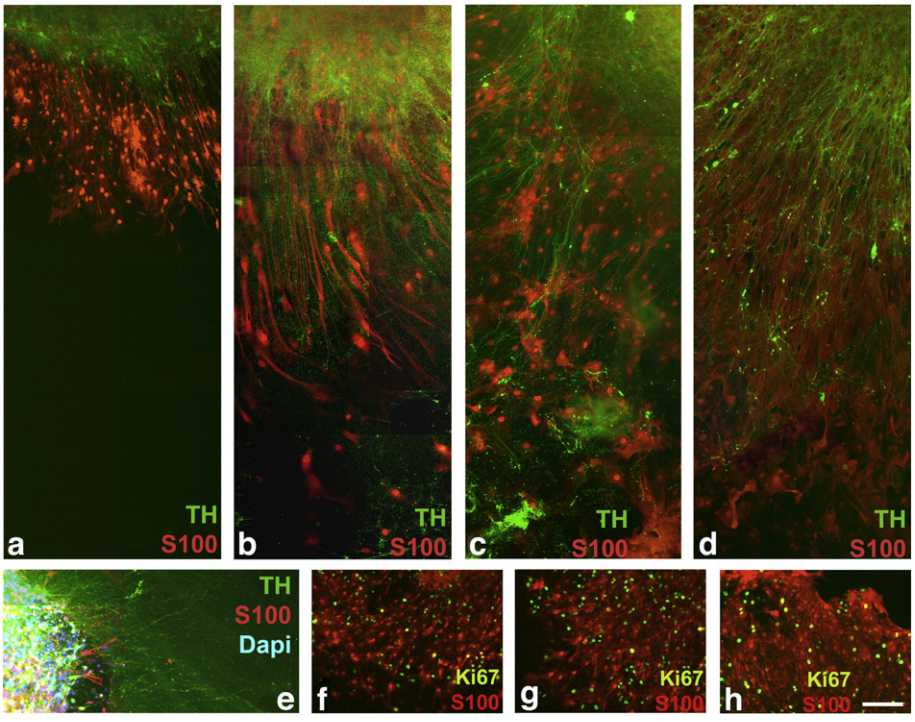

Fig. 1 – Dopamine nerve fiber outgrowth after treatment with TNFα. Dopamine neurons were visualized using tyrosine hydroxylase (TH) and astrocytes were visualized using S100, for cell nuclei staining, DAPI was used in VM organotypic tissue cultures. The VM cultures were treated with TNFα between days 4 and 7 (b) or between days 11 and 19 (d) and fixed either on day 7 (a, b) or day 19 (c, d). The glial-guided TH-positive outgrowth is enhanced by the early treatment (b), and the astrocytic migration reached longer distances from the tissue slice compared to that measured in the control cultures at 7 DIV (a). TNFα treatment of VM cultures evaluated at 19 DIV (d) did neither demonstrate a difference for the glial-guided outgrowth nor for the astrocytic migration compared to control at day 19 (c). The non-glial-associated TH-positive outgrowth was determined by the absence of glial cells, seeing by the negativity to DAPI (e). In cultures processed for the astrocytic marker S100 and the proliferation marker Ki67 (f–h), the number of Ki67-positive cells did not differ between the control (f), cultures treated with TNFα (g), or TNFα plus antibodies against TNFR1 (h). Scale bar: a–d = 100 μm, e–h = 150 μm.