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Trimethyltin-induced plastic neuronal changes in rat hippocampus are accompanied by astrocytic trophic activity

Danuta Koczyk, Małgorzata Skup, Małgorzata Zaremba and **Barbara Oderfeld-Nowak**[1]

Department of Neurophysiology, Nencki Institute of Experimental Biology, 3 Pasteur St., 02-093 Warsaw, Poland

Abstract. Partial deafferentation of the hippocampus due to trimethyltin (TMT) intoxication has been reported to induce plastic rearrangements of neuronal elements but the factors that direct these responses are unknown. To assess the possible involvement of nerve growth factor (NGF) in the phenomenon we evaluated the presumable changes in the expression pattern of NGF immunoreactivity (NGF-IR) in rat hippocampus 21 days after administration of TMT (8 mg/kg, i.p.) when reactive changes are fully developed. Immunolabelling for TrkA known to mediate biological effects of NGF and for GFAP to identify astroglial cells as a one of presumed source of postinjury produced factors was carried out on adjacent sections to establish the relation between expression of these proteins. In control hippocampus NGF- IR and TrkA-IR were localized in neurons and/or neuropil. After exposure to TMT remarkable non-neuronal expression of both proteins was observed. The distribution pattern of NGF, TrkA and GFAP overlapped suggesting that reactive astrocytes may not only produce NGF but also may become responsive to this neurotrophin. Zones of extensive NGF and TrkA astroglial expression corresponded to areas of axonal-dendritic rearrangements reported earlier. The data suggest that astroglia-derived trophic activity may be involved in neuronal plastic events associated with treatment with TMT.

Short communication

[1]To whom correspondence should be addressed

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Due to the characteristic pattern of damage produced by peripheral administration of trimethyltin (TMT) to rat, this compound may be used as a model neurotoxicant for investigating changes subsequent to neuronal injury. Although TMT-induced degeneration is widespread within the central nervous system the hippocampus is preferentially affected by the toxin (Balaban et al. 1988, Whittington et al. 1989). TMT intoxication results in pyramidal cell loss in CA4/CA3 and CA1 subfields and the destruction of the perforant path, a major input to the hippocampus from the entorhinal cortex, which leads to denervation of the distal dendrites of granule cells.

Neuronal loss and terminal axonal degeneration are followed by an increase in the hippocampal cholinergic markers as revealed by measurements of choline acetyltransferase (ChAT) activity (Cannon et al. 1995) and acetylcholinesterase (AChE) histochemical staining (Woodruff and Baisden 1990), and in the concentration of synaptic vesicle-associated proteins, synapsin I and p38 (Brock and O'Callaghan 1987). These results indicate replacement of degenerated fibres by sprouted intact afferents from the medial septum, normally terminating in the hippocampus in a rather diffusible fashion. Apart from alterations in axonal distribution and synapse reorganization, transneuronal changes on dendrites of denervated granule cells may occur. The latter have been predicted from our immunocytochemical studies on the fate of dendrite-related microtubule-associated protein, MAP-2 (Koczyk 1994). Together, these data suggest that both pre- and post-synaptic elements participate in structural rearrangements due to exposure to TMT.

Although the mechanisms underlying plastic responses remain unknown, the possible role of trophic factors has been suggested. Nerve growth factor (NGF) seems to be a potential candidate to mediate TMT-induced structural alterations, since numerous studies have implicated this neurotrophin in sprouting response (see Crutcher 1987) and it is well established that, within the CNS, NGF is a target-derived trophic factor for the cholinergic neurones of the basal forebrain eliciting in them a variety of biochemical as well as morphological changes (Higgins et al. 1989, Gage et al. 1990). TMT intoxication is accompanied by a marked glial reaction (Brock and O'Callaghan 1987, our unpublished observations) and very recent reports, including ours, indicate that upon injury reactive astrocytes may become a source of NGF (for review see Oderfeld-Nowak and Bacia 1994). We therefore hypothetized that after exposure to TMT hippocampal glial cells may acquire the capacity to produce NGF, and our preliminary observations supported this possibility (Oderfeld-Nowak et al. 1995).

In the present study we examined the presumed changes in the expression of NGF immunoreactivity in different cell types in the hippocampus after exposure to TMT. Since NGF exerts its trophic activity *via* high-affinity receptor, TrkA, (for review see Barbacid 1994), immunocytochemical expression of this protein was also evaluated. Analysis was performed on 21 day after neurotoxin administration because hippocampal neuronal loss, reactive gliosis and axonal-dendritic restructuring are significantly advanced at this postexposure time-point.

Adult male Wistar rats (3 months old) were used. Experimental animals received a single intraperitoneal injection of TMT chloride (8 mg/kg; Merck) in phosphate-buffered saline (PBS). PBS-treated rats served as controls. After administration the animals were allowed to survive for 21 days, were then perfused transcardially with 0.01 M PBS + heparin (6250 U/l) followed by 2% paraformaldehyde + 0.2% parabenzoquinone in 0.01 M PBS. Brains were removed, postfixed in the same fixative and cryoprotected in 30% sucrose in 0.1 M PBS for 48 h at 4°C. Free-floating sections (25 μm) were processed for immunocytochemistry using a standard avidin/biotin procedure. Sections were incubated with primary antibodies (overnight at 4°C): polyclonal rabbit anti-NGF (1:200; kindly provided by Prof. L. Aloe, Institute of Neurobiology, Rome), polyclonal rabbit anti-TrkA (1:250; Santa Cruz), and monoclonal mouse anti-glial fibrillary acidic protein (anti-GFAP) to identify astrocytes (1:1000, Boehringer). Further steps were performed by application of secondary antibodies ((1:200; ABC Kit,

Vector Labs.) (1 h, room temperature)), followed by incubation with an avidin/biotin-peroxidase reagent ((1:100; ABC Kit) (1 h, room temperature)) and by development of enzymatic reaction with 0.2% 3,3'-diaminobenzidine tetrahydrochloride (DAB) + 0.02% H_2O_2. Control sections were processed similarly using washing buffer (PBS + 0.3% Triton X-100) in place of the primary antibody.

In the hippocampal sections from control rats NGF- and TrkA-immunoreactivity was found to be localized exlusively in neurones and/or neuropil, confirming our previous observations (Bacia et al. 1992, Skup et al. 1995). The pattern of distribution of NGF and TrkA in CA1 subfield is shown in Fig. 1A and B. Within 21 days after treatment with TMT, striking alterations in expression patterns of both proteins occurred. For NGF, decreased neuronal immunolabelling was observed, most probably due to the loss of pyramidal cells (see CA1 pyramidal layer, Py, in Fig. 1D and compare with Fig. 1A) as was expected from studies on TMT-induced degeneration observed by us (not shown). On the other

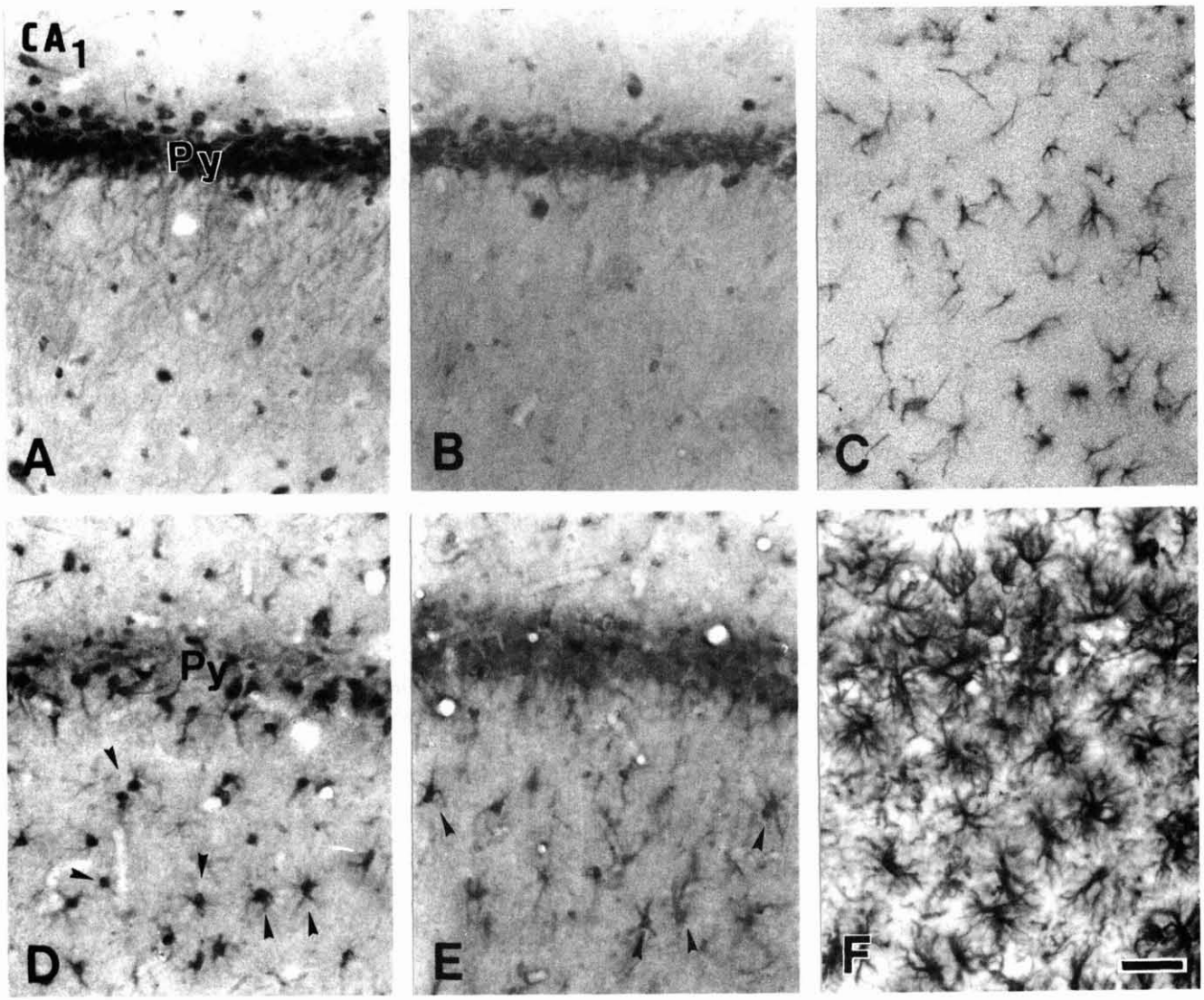

Fig. 1. TMT-induced astroglial appearance of trophic activity on 21 day after exposure. Upper panel: adjacent sections of control hippocampal CA1 subfield stained for NGF (A), TrkA (B) and GFAP (C). Lower panel: NGF-IR (D) and TrkA-IR (E) in astrocyte-like cells (arrowheads) after treatment with TMT. Note reactive astrocytes in the same field (F). Py, pyramidal layer. Scale bar = 40 μm.

hand, many cells of astrocytic appearance stained for NGF were found in lesioned areas (exemplified by CA1 subfield in Fig. 1D, arrowheads) and, to a lesser extent, in the denervated zone (not shown). While localization of TrkA in fibre terminals seemed to be unchanged at that time-point (not shown), there was a robust non-neuronal expression of the protein, suggesting its appearance in astroglia (Fig. 1E, arrowheads). Comparison of the distribution of NGF- and TrkA-positive glial cells with GFAP-stained astrocytes responding to treatment with TMT (GFAP-IR is shown in Fig. 1C and F) in adjacent sections demonstrated that the fields of cells expressing all three studied proteins overlapped (Fig. 1D, E and F).

Our results demonstrate that hippocampal astroglial cells express trophic activity after exposure to TMT. These data are in agreement with other *in vivo* studies showing that upon injury reactive astrocytes express NGF (Bakhit et al. 1991, for references see Oderfeld-Nowak and Bacia 1994). They also add to the single reports on TrkA expression in activated astrocytes (e.g. Junier et al. 1994). Furthermore, the astrocytic expression of NGF and TrkA exhibits both spatial and temporal correspondence not only to neuronal degeneration (Whittington et al. 1989, our observations) but also to axonal-dendritic remodelling (Koczyk 1994, Cannon et al. 1995). The data suggest the involvement of glial-derived NGF in the process of structural reorganization of hippocampal connections. Whether neurite-stimulating properties of this neurotrophin contribute to compensatory or aberrant changes in neuronal morphology remains to be elucidated.

The appearance of TrkA on astrocytes observed by us suggest that glial cells themselves may synthetize this receptor protein. Neurotrophin receptors are known to be upregulated by their respective ligands (Holtzman et al. 1992), thus suggesting that TMT-induced TrkA astroglial expression may occur *via* NGF-dependent mechanism. TrkA upregulation might be a part of a mechanism enabling glial cells to respond to localized release of NGF. Our study indicates that reactive astrocytes may not only be the producers of neurotrophins but also their targets and speaks in favour of NGF autocrine/paracrine mode of action. Alternatively, TrkA receptors may serve as scavengers of astroglia- and neurone--released NGF contributing to the increase of its local concentration that, in turn, may attract fibre terminals.

In conclusion, our data suggest that after exposure to TMT a switching from neuronal to non--neuronal expression of NGF occurs. Whether bioavailability of astrocyte-derived NGF is sufficient to promote structural changes induced by the toxin and whether these changes are in fact driven by astroglial NGF is still an open question.

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