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Differential response of microtubule-associated protein 2 (MAP-2) in rat hippocampus after exposure to trimethyltin (TMT): an immunocytochemical study

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Abstract. Several neurotoxins induce changes in microtubule-associated protein 2 (MAP-2), the cytoskeletal protein primarily and highly enriched in the dendritic compartment of neurones. The present study aimed to investigate the fate of MAP-2 after administration of trimethyltin (TMT), an environmental neurotoxin. An immunocytochemical staining was performed in the hippocampus, known to be the most vulnerable brain region after TMT exposure. Prolonged survival time (21 days) following i.p. injection of a single dose of TMT (8 mg/kg) led to a considerable changes in intensity and pattern of distribution of MAP-2 immunoreactivity within the structure. A significant decrease in the staining was observed in the hippocampus proper especially in CA4/CA3 and CA1 subfields. This decrease was correlated with the severity in pyramidal cell loss previously reported by others. On the contrary, an increased density of MAP-2 immunostained dendrites was found in the molecular layer of dentate gyrus. Since TMT has been recognized as an agent damaging not only the hippocampus but also other limbic structures, the latter result might be interpreted in terms of postsynaptic changes due to hippocampal deafferentation.

Short communication

Key words: trimethyltin, microtubule-associated protein 2, hippocampus, dentate gyrus, deafferentation

Trimethyltin (TMT) is one of several organotin compounds of industrial, commercial and environmental importance. Toxicity of these compounds has been recognized for many years. TMT produces behavioural and neuropathological symptoms in humans (Boyer 1989) and experimental animals (Dyer et al. 1982, Boyer 1989, Earley et al. 1992). A single systemic exposure of rat to TMT causes necrosis of neurones in some regions of the brain but has an especially severe effect on the hippocampus (Chang and Dyer 1983, Balaban et al. 1988).

Microtubule-associated protein 2 (MAP-2), one of the major components of brain microtubules, is primarily and highly concentrated in dendrites and maintains their integrity (Lewis et al. 1989). Some evidence indicate that this cytoskeletal protein may dynamically change in response to many inputs (for review see Johnson and Jope 1992). There are also data to show that immunocytochemistry, concentration and phosphorylation state of MAP-2 may be modified by different neurotoxins, including aluminum, tri-o-cresyl phosphate, N-methyl-D-aspartate, kainate (Johnson and Jope 1992).

It thus appeared of interest to assess whether MAP-2 is a TMT target as well. This study was designed to investigate this issue using immunocytochemistry for MAP-2. A preliminary report of the study appeared in abstract form (Koczyk et al. 1993).

Adult male Wistar rats (240-270g) were treated with a single i.p. dose of 8 mg/kg TMT chloride (Merck) in phosphate-buffered saline (PBS) (n=4) or PBS alone (n=2). Animals were killed by decapitation on day 21 postexposure. Fresh brains were removed from the skull and fixed in 4% paraformaldehyde for 4 days and subsequently transferred to 30% sucrose until sectioning. Free-floating 25 µm sections were used. Endogenous peroxidase activity was blocked by immersion in 0.3% H_2O_2 in methanol for 20 min, then 5% horse normal serum was applied to the sections for 30 min. Subsequently, sections were processed for immunocytochemistry using a biotin-avidin immunoperoxidase system (Vectastain ABC Kit, Vector Labs., Inc.). A monoclonal mouse anti-MAP-2 (Sigma) antibody was used at the dilution 1:1500 for a period of 24 h (4°C). That preceded incubation with a biotinylated anti-mouse IgG (1:200, 1 h), followed by incubation with avidin-biotinylated peroxidase complex (1:200, 1 h) before visualization of the complex with 3,3'-diaminobenzidine (0.2%; Sigma) in the presence of H_2O_2 (0.02%). Sections treated with PBS rather than primary antibody were used as controls. Sections were mounted on glass slides and examined under the light microscope.

Figure 1A shows the immunocytochemical staining of the control (PBS treated) hippocampus. MAP-2 was present both in somata and dendrites but perikarya of the pyramidal and granular cells stained weaker than dendritic processes. MAP-2 immunostaining of dendritic tree was rather evenly distributed within the subfields of the hippocampus proper and the molecular layer (ML) of dentate gyrus. Higher magnification of DG is presented on Fig. 1A'.

Intensity and pattern of distribution of MAP-2 immunoreactivity (MAP-2-IR) dramatically changed in the hippocampus three weeks after exposure to TMT (Fig. 1B). A significant decrease in MAP-2-IR in CA1 (well visible remaining dendrites in contrast to the control where the staining was much more denser) and almost a complete loss of reaction in CA4/CA3 hippocampal areas were observed (Fig. 1B, arrows). In contrast, as compared to PBS treated animals, the molecular layer of dentate gyrus showed an increased MAP-2 immunostaining (Fig. 1B, 1B', arrowhead). The intensity of staining was homogenous throughout this layer.

The present data indicate that there is a differential response of MAP-2 assessed by immunocytochemistry in the hippocampus at prolonged survival time after exposure to TMT: a decreased or even lost staining in CA1 and CA4/CA3 and an increased immunoreactivity in dentate gyrus. A close correspondence was found between the sites of TMT-induced damage of pyramidal cells previously described (Balaban et al. 1988) and the regional pattern of decreased or lost expression of MAP-2 observed in this study. Given the lack of changes in the distribution of the protein at early postlesion inter-

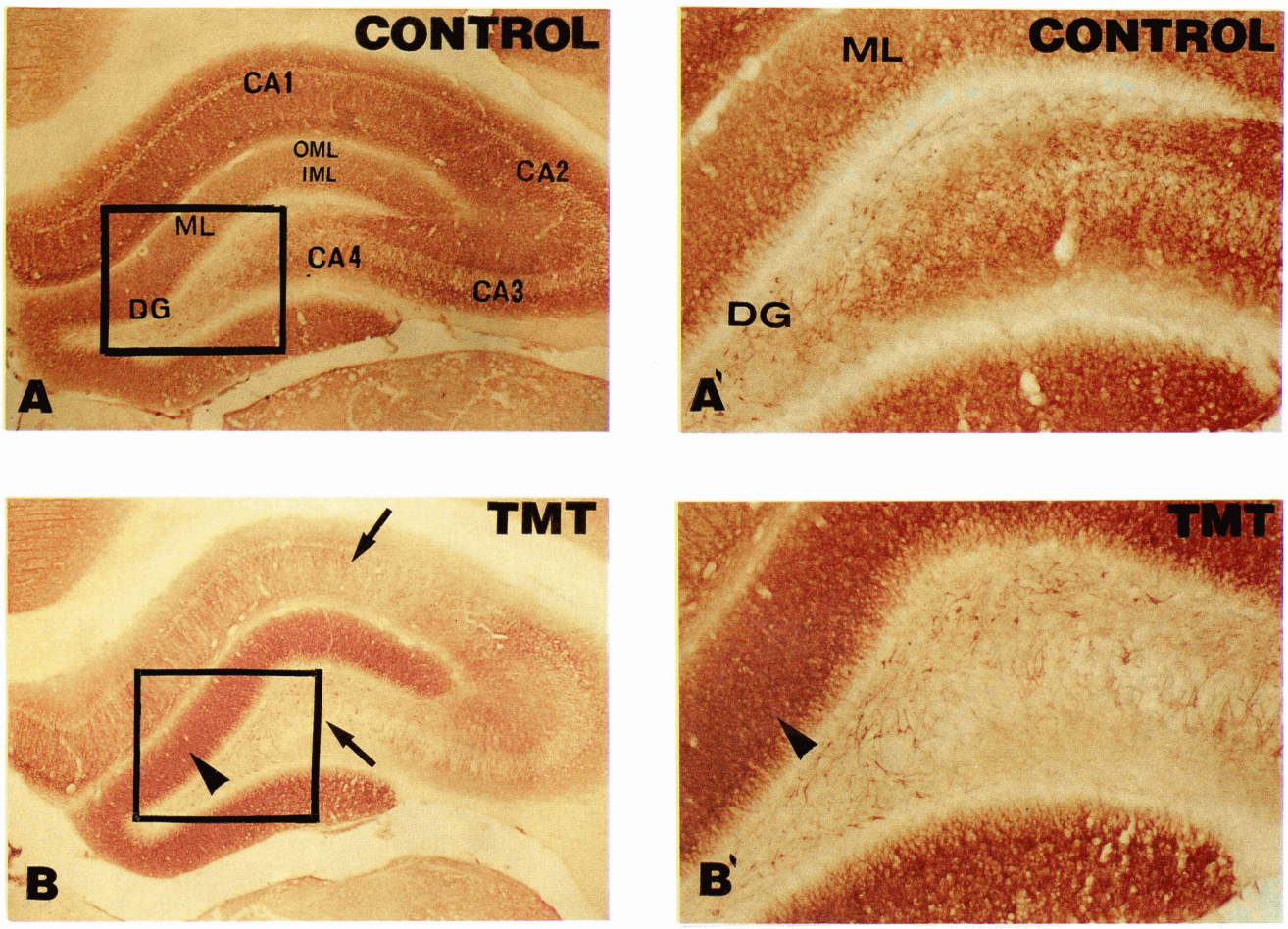

Fig.1. Immunocytochemical staining of MAP-2 on day 21 in the hippocampus of PBS-treated rat (A) and of a rat exposed to 8 mg/kg TMT (B). (A') and (B'), higher magnification of DG of PBS- and TMT-treated rats, respectively. Significantly decreased or even lost immunoreactivity was found in CA1 and CA4/CA3 hippocampal areas (B, arrows). An increased homogenous immunostaining was observed along the whole extent of ML of DG (B, B', arrowhead). Original magnification for (A,B), 8x and for (A', B'), 20x. CA1, CA2, CA3, CA4, subfields of the hippocampus proper; DG, dentate gyrus; ML, molecular layer of dentate gyrus; OML, outer molecular layer; IML, inner molecular layer.

vals found by us (Koczyk et al., in preparation) as well as histological evidence of hippocampal impairment reaching a maximum within 21 days following TMT exposure (Earley et al. 1992), it may be suggested that loss of MAP-2-IR reflects most probably massive loss of pyramidal cells in the affected regions. Undoubtedly, an increase in MAP-2-IR within the molecular layer of dentate gyrus represents even more interesting finding of the current study. In relation to this point several aspects deserve consideration. Although hippocampal lesion is widely reported and highly visible, TMT-induced damage may be widespread. With the used dose other brain structures are affected too, especially pyriform/entorhinal cortex (Balaban et al. 1988) whose destruction is evident three weeks postexposure. It has been previously shown that surgical lesions of the entorhinal cortex (EC), which provides the outer molecular layer (OML) of DG with perforant path afferents, lead to postlesion reorganization of inputs within the denervated ML (for review see Cotman and Nadler 1978). Such a type of denervation is also capable of inducing considerable changes in microtubule-associated pro-

tein 2 (Caceres et al. 1988). It has been suggested that a redistribution of MAP-2 from non-denervated regions of the dendritic tree towards the denervated ones is responsible for an increase in MAP-2-IR in the outer molecular layer during the early postlesion interval followed by a general increase in the staining within the whole layer (Caceres et al. 1988). This transient increase in MAP-2 reactivity may be related to the remodelling of dendrites (Caceres et al. 1988). Since TMT administration causes the perforant path to degenerate (Balaban et al. 1988) leading to hippocampal deafferentation, it seems possible that certain changes in the microtubule protein associated with the dendritic reorganization may exist in DG in this model as well. It should be noted here that the pattern of MAP-2 staining in DG subsequent to exposure to TMT does not exactly mimic the effects of surgical lesion of the EC and that the result is probably a consequence of the specificity of the TMT model. Granule cell dendrites responded in a uniform pattern of an increased MAP-2-IR and no differences in the staining within the whole ML were observed neither three weeks postexposure nor at early survival times (Koczyk et al., in preparation). TMT-induced destruction of CA4/CA3 pyramidal neurones evokes deafferentation of their terminal field, i.e. the inner molecular layer (IML), a phenomenon which seems to parallel in time deafferentation of the OML following intoxication (Balaban et al. 1988). Putative parallelism of changes in MAP-2 in the IML and OML due to concurrent deafferentation of both zones may give rise to the homogenous staining throughout the dendrites. With all that in mind, it is difficult to explain the increase in MAP-2-IR observed in the ML following toxin administration in terms of redistribution of the protein between non- and denervated zones: the OML as well as the IML are both directly denervated. Synthesis of the protein preceding synaptic replacement would be an attractive explanation. Although no quantitative analysis has been performed, it does not seem that an increase in MAP-2-IR along the whole extent of the ML results from the shrinkage of the layer.

Thus, the observed increase in MAP-2-IR in DG may suggest that changes in cytoskeletal protein are related to the lack of afferent system and postsynaptic response of denervated neurones rather than a direct action of neurotoxin. Further studies are necessary to prove or disprove this hypothesis.

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