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How does trimethyltin affect the brain: facts and hypotheses

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Abstract. Trimethyltin, an organic compound of tin, is a potent neurotoxicant of a mechanism of action yet to be uncovered. The neuropathological findings that trimethyltin causes selective hippocampal damage with several unique features, highly reminiscent of Ammon's horn sclerosis as a final result, have raised the possibility that there is a link between trimethyltin neurotoxicity and other degenerative events for which an imbalance between neuronal inhibition/excitation has been proposed. However, there still exists a whole catalog of issues which await clarification. One of the greatest importance is how does trimethyltin reach the critical sites within the brain and what are they? Available data concerning the long-term consequences related to trimethyltin neurotoxicity are also far from being completed. This review summarizes current data from *in vitro* and *in vivo* studies on neurotoxic effects of trimethyltin. Several hypotheses on mechanisms that may lead to neuronal death induced by the toxin are presented.

Key words: trimethyltin, TMT, neurotoxicity, selective vulnerability, excitotoxicity, plasticity, hippocampus

Review

INTRODUCTION

Organotins have had widespread use in both industrial and agricultural settings (Boyer 1989). As far back as 1880 toxic properties of these compounds were recognized (White 1880). Lower trialkyltins affect the central nervous system and several poisoning incidents have been documented (Barnes and Stoner 1959, Fortemps et al. 1978, Ross et al. 1981, Feldman et al. 1993). Trimethyltin (TMT) is an intermediate by-product in the production of other more commonly used tin compounds. It thus still constitutes an occupational hazard for some groups. However, much of the recent interest in this compound comes not from the standpoint of environmental toxicology but rather from the possibility that TMT might become important research tool for the study of brain function.

By far the most studies on TMT-induced toxicity were performed with rats. These animals peripherally given TMT at high doses (6-9 mg/kg) reveal a remarkably similar behavioural and neuropathological profiles to those reported in human victims (see Woodruff and Baisden 1994). Behavioural, neuropathological and neurochemical effects resulting from TMT intoxication of rats are the subject of this review. Emphasis of the article is directed especially to provide an overview on proposed mechanisms of TMT action and on adaptive processes induced by the toxin.

BEHAVIOUR AND NEUROPATHOLOGY

Rats exposed to TMT demonstrate tremors, seizures, tail mutilation and irritability. Such an acute "TMT syndrom" (Dyer et al. 1982a) begins within 2-3 days following treatment and largely disappears 2-3 weeks postexposure but animals continue to show hyperactivity (Ruppert et al. 1982, Cannon et al. 1992), disrupted patterns of self-grooming (Cannon et al. 1992), deficits in radial-arm and water-maze acquisition (Walsh et al. 1982b, Woodruff et al. 1991), impaired performance of the differential reinforcement of low response rates (DRL) operant schedule (Mastropaolo et al. 1984, Woodruff et al. 1991), and impaired acquisition of passive avoidance (Walsh et al. 1982a). The constelation of both acute and chronic behavioural effects is probably attributable to limbic system pathology.

TMT intoxication results in neuronal cell death; degenerative changes do not become evident immediately postexposure. For example, in the hippocampus which is the most affected structure damage is observed as early as 2 days after a single treatment and progresses over time becoming evident within 21 days (Brock and O'Callaghan 1987) and continues during several next weeks (Whittington et al. 1989). The delayed onset and prolonged duration are likely due to the high affinity of rat hemoglobin for TMT (Rose and Aldridge 1968). Hemoglobin may therefore serve as a reservoir slowly and continously releasing TMT into the plasma from which it then enters the brain.

As has been stressed, the primary target for TMT is the hippocampus. While there is a general agreement that TMT exerts its toxic effects on pyramidal neurones, especially in CA4/CA3 and CA1 subfields (Chang and Dyer 1983, Balaban et al. 1988, Whittington et al. 1989), histological examination of granule cells revealed contradictory results. Some investigators observed granule cell loss that subsided relatively early postexposure (Whittington et al. 1989); others saw no degenerative changes in these cells at all (O'Callaghan and Miller 1984). This discrepancy most probably stems from the differences in experimental conditions. In this regard, several factors should be considered: species of rat, time-points investigated, staining methods, i.e. cresyl violet vs. silver stains, and the hippocampal plane studied. As far the latter is concerned, the study by Chang and Dyer (1985) shows that the vulnerability of the hippocampus to TMT is differentiated along its septotemporal axis. This observation has recently been confirmed by Whittington et al. (1989). Accordingly, dentate granule cells are most involved temporally and are only minimally affected at the septal pole. However, regardless of the regional specificity the majority of these cells seem to sur-

vive exposure to the toxin. This suggestion is compatible with those of Chang et al. (see Chang 1984, 1986) and Sloviter et al. (1986) that intact granule cells are necessary for the destruction of pyramidal neurones. In support, Theoret and Krigman (1985) reported that TMT-induced CA4/CA3 damage can be prevented by eliminating the granule cells. Also data obtained by Koczyk (1994) argue for that gross granule cells are resistant to TMT (see below).

MECHANISMS CONTRIBUTING TO TMT-INDUCED BRAIN DAMAGE

Although TMT has been recognized as a hippocampal neurotoxin later studies have indicated that its central effects are more widespread (Balaban et al. 1988). The data do not imply a lack of selectivity of the toxin for particular populations of neurones. Specific, highly consistent patterns of cell death were observed in various brain structures indicating that the effects are selective. Explaining selective vulnerability of a brain region to a neurotoxicant requires addressing the question of whether the neurotoxicant is sequestered in the vulnerable area in greater concentration or for a longer time period than in other brain areas, or whether the cells in the affected region are more sensitive to the neurotoxicant.

Addressing the first issue it has to be stated that the selective nature of the lesion cannot be explained by a preferential distribution of TMT or tin to sensitive areas (Mushak et al. 1982, Cook et al. 1984a); moreover, elemental tin and other metabolites of TMT do not display this pattern of neurotoxicity (Cook et al. 1984b, Boyer 1989).

The factors that predispose cells to the neurotoxic actions of the organotin are not well characterized. At the molecular level, studies of Billingsley and his group (Krady et al. 1990, Toggas et al. 1992) are the only ones in the area. By coupling subtractive hybridization with molecular cloning techniques, these authors isolated a cDNA specifically localized in TMT-sensitive cells. This cDNA encodes a putative peptide termed "stannin" (from the Latin *stannum* which means related to tin). In immunocytochemical experiments carried out by these groups antisera raised against the stannin exhibited strong immunoreactivity in TMT-sensitive neurones in the hippocampus and entorhinal cortex, areas previously labelled with stannin coding sequences. The authors suggest that stannin may play a role in the selective toxicity of TMT. The identity of the protein and its activity remains to be established.

The biochemical changes that follow *in vivo* and *in vitro* exposure to TMT have been demonstrated in a number of studies, yet their contribution to neuronal cell death are not understood. These primarily include interference with cholinergic, GABAergic and glutamatergic systems. Since neurotoxin-induced dysfunction in these systems has been suggested to be involved in TMT-induced pathology, it will be described in some detail.

Earley et al. (1989) and Conner et al. (1994) using *in vitro* autoradiography described a large reduction in muscarinic receptor binding in the hippocampus following TMT treatment. It has been shown that the decrease, most probably reflecting neuronal loss, involves both the M1 and M2 subtypes (O'Connell et al. 1994b) and may be successfully prevented by tacrine, a potent reversible cholinesterase inhibitor (O'Connell et al. 1994c). Tacrine treatment also resulted in a significant improvement in the Morris water maze performance (O'Connell et al. 1994c). So far the basis of protective effect of tacrine has not been understood; both direct (i.e. interaction with cholinergic neurotransmission) (Nordberg et al. 1988, Hunter et al. 1989) as well as indirect (i.e. interaction with glutamatergic system through phencyclidine (PCP) site situated on the ion channel of the N-methyl-D-aspartate (NMDA) receptor complex) (Davenport et al. 1988) mechanism of action has been suggested. While the aforementioned results provide evidence for the involvement of cholinergic mechanisms in TMT-induced cognitive deficits in particular, the enhanced activity of cholinergic markers, acetylcholinesterase (Woodruff and Baisden 1990) and

choline acetyltransferase (Cannon et al. 1995) may be explained in terms of structural plasticity evoked by TMT intoxication (see below). Thus, axonal sprouting of the cholinergic projection to the hippocampus does not seem to be reflected by preservation of the muscarinic receptors, especially M2 subtype thought to be localized mainly presynaptically (Vogt 1988).

TMT exposure reduces the concentration of GABA within the hippocampus (Wilson et al. 1986, Earley et al. 1992) suggesting that TMT affects the GABAergic system. It is not clear whether this reduction is due to a loss of the GABA-utilizing basket cells or changes in their functional state. Since it has been shown that exposure to the toxin reduces inhibition within the dentate gyrus (Dyer et al. 1982b, Dyer and Boyes 1984, Janigro and Costa 1987) but does not provoke changes in the activity of glutamic acid decarboxylase (GAD) (Naalsund et al. 1985), a specific marker for GABAergic synapses, and decreases the high affinity uptake of the neurotransmitter (Doctor et al. 1982, Valdes et al. 1983, Naalsund and Fonnum 1986b) the second possibility seems to be more likely. The decreased GABA levels together with altered activity of basket cells may be the mechanism which reduces the recurrent inhibition involving the dentate granule cells. The concept of "functional toxicity" was introduced by Chang and coworkers (see Chang 1986) to indicate that neurone specific damage in one region may be the result of adverse alteration of the functional state of neuronal cells in another. It has been proposed that TMT may act on the GABAergic inhibitory system of the hippocampus to elicit a hyperexcitatory state of the dentate granule cells and produce hyperstimulatory damage to their CA4/CA3 pyramidal target neurones. Recent observations of O'Connell and coworkers (1994a) that the $GABA_A$ receptor agonist, gaboxadol, improves TMT-induced spatial navigation deficits further support the hypothesis that TMT may exert its neurotoxic effect by antagonizing GABAergic system.

Because TMT produces selective hippocampal lesion similar to that caused by convulsants which interact with the brain excitatory amino acid transmission (compare to neuropathological pattern evoked by kainic acid (KA), an exogenous agonist of a subclass of excitatory amino acid receptors (EAA), see Ben-Ari 1985) the hippocampal glutamate system was studied by several groups in order to evaluate its possible contribution to neuronal damage produced by TMT. Naalsund and Fonnum (1986b) found that TMT exposure is accompanied by a decrease in glutamate levels. This finding is consistent with increased glutamate release coupled with the blockade of its reuptake reported for synaptosomal preparation observed by the same authors (1986b). Since it has been shown that organotin compounds induce several effects on membrane ion transport system, including Cl^-/OH^- exchange (Selwyn et al. 1970) and a reduction in the extracellular Cl^- levels is known to induce a glutamate release *in vitro* (Naalsund and Fonnum 1986a), a TMT-induced chronic increased efflux of glutamate might be due to an alteration in external Cl^- concentration. The observations of Naalsund and Fonnum (1986b) are in agreement with those of Patel et al. (1990) who found that in partially depolarized hippocampal slices TMT produces a release of endogenous glutamate through a calcium-dependent process.

Unlike KA, TMT does not appear to be excitatory in nature (Allen and Fonnum 1984, Dyer and Boyes 1984) but similarity of final hippocampal damage produced by both toxins implies that neuronal death may be due to excessive depolarization evoked by excitatory substances. Based on the results coming from their own comparative studies Sloviter et al. (1986) proposed that the TMT-induced damage is in part mediated by seizure activity which is probably due to the release of "endogenous excitotoxin". However, the pathways in which seizure activity is induced are probably different in both cases (Sloviter et al. 1986). Little information is available on how TMT can produce seizure activity *in vivo*. One possibility is that TMT interacts with zinc in the presynaptic terminals of excitatory fibres that in turn may affect transmitter-dependent processes. Consistent with this hypothesis is the finding by Chang and Dyer (1984) that TMT de-

creased histochemically stainable metal in the hippocampus before seizures or damage occur.

The proposal of the excitatory mechanism of TMT action implies the involvement of EAA receptors. Reversal of the behavioural deficits in TMT--treated rats after PCP treatment has been observed by Earley et al. (1990). These findings coupled with reported non-competitive blockade of NMDA responses by drugs active at the PCP site (Freeman and Dawson 1991) suggest that some of the neurotoxic effects of TMT may be mediated by NMDA/PCP receptor complex. The contribution of NMDA receptors to TMT toxicity has also been suggested by Aschner and Aschner (1992) (see below). Thus, the possibility exists that some of the hippocampal cell loss evoked by TMT treatment results from release of excitotoxin acting *via* NMDA receptors. However, the regional distribution of high concentrations of these receptors does not correlate with cell loss across all brain regions since areas such as the molecular layer (ML) of the dentate gyrus that possess high concentration of NMDA receptors (Monaghan and Cotman 1985) are relatively well preserved (see above). The properties of neurones themselves, and perhaps their ability to regulate intracellular Ca^{2+} may also play a role (but see also Oyama et al. 1993).

The involvement of metabotropic receptors in TMT toxicity has been postulated by Pavlaković and coworkers (1995). Very recent report of these authors demonstrates that TMT leads to a sustained protein kinase C (PKC) activation/translocation from cytosolic to the plasma membrane in differentiated PC12 cells. Inhibition of PKC with chelerythrine or down regulation of the enzyme by phorbol myristate provided partial protection against TMT--induced cell injury. The influence of TMT on PKC does not seem to be associated with an elevated cytosolic Ca^{2+} concentration, since it has been shown by Oyama et al. (1993) that in cerebellar granule cells TMT treatment does not contribute to such an increase. Discussing the possible mechanism leading to the activation of PKC the authors point to that TMT-induced increases in extracellular glutamate (see above) may be responsible for activation of phospholipase C- and phospholipase A_2-coupled receptors which subsequently generate arachidonic acid (Käfer et al. 1992), and possibly diacylglycerol (see Pavlaković et al. 1995), both activators of PKC even in the presence of normal Ca^{2+} concentration (Kikkawa et al. 1982).

Recent *in vitro* studies show that TMT exerts its toxic effects directly on glial cells. The interaction of TMT with rat brain type 1 astrocytes *in vitro* has been investigated by Richter-Landsberg and Besser (1994). The data from this study indicate that TMT--induced cytotoxic effects include changes in cell morphology, disturbance of the intermediate filament system and an increase in levels of glial fibrillary acidic protein (GFAP). These effects have also been described after *in vivo* administration of TMT (see Brock and O'Callaghan 1987, O'Callaghan 1993, Andersson et al. 1994) but the data of Richter--Landsberg and Besser demonstrate that at least *in vitro* reaction of astrocytes to the toxin is independent on neuronal signals. It is likely that the disturbance of the filament system is due to an increased activity of PKC; GFAP is a substrate for PKC (Harrison and Mobley 1991) and phosphorylation of the protein *in vitro* (Inagaki et al. 1990) results in a disassembly of the filament structure. The study of Richter-Landsberg and Besser also revealed that TMT treatment led to a remarkable decrease in the activity of ecto-5'-nucleotidase which in addition to its enzymatic function (Kreutzberg 1986) represents a transmembrane cell surface protein that may participate in cell communication and in the interaction between cells and their external environment in particular (Olmo 1992).

Using primary cultures of astrocytes Aschner et al. (1992) observed TMT-induced changes in Na^+-K^+-ATPase activity and ion permeability resulting in astrocytic swelling. Other findings from these studies demonstrate that TMT inhibits the Na^+-dependent L-glutamate and D-aspartate uptake and increases the rate and the amount of glutamate efflux from astrocytes in a dose-dependent fashion. On the basis of these results coupled with previous reports on depression of mitochondrial respiration by TMT (Aldridge 1958, Stockdale et al. 1970, Nicklin and

Robson 1988), Aschner and Aschner (1992) proposed the toxin-mediated mechanism of neuronal injury. TMT would lower the neuronal resting membrane potential to a point where the voltage--dependent Mg^{2+} blockade of the NMDA receptor is released enabling the excitatory amino acids (liberated from swollen astrocytes from glutamate pool) to act persistently on the neurone increasing calcium permeability through the ion channel complex and triggering a degenerative cascade (Novelli et al. 1988). The suggestion has been added that as brain astrocytes are thought to form a heterogenous population of cells (for references see Aschner and Aschner 1992), a predilection of these cells to TMT may account for the regional specificity of TMT toxicity.

Using aggregating brain cell cultures Monet--Tschudi et al. (1995) showed that microglial cells were the first to respond to TMT treatment. The reaction revealed by immunocytochemical staining occurred at TMT concentration where no changes in either neuronal or astroglial parameters could be detected. This study limits the resolution whether microglia respond directly or indirectly to TMT. But given the potential of microglial cells to mediate damage of other cell types due to release of toxic substances such as nitric oxide, glutamate and cytokines (see Banati et al. 1993), their contribution to the changes induced by TMT *in vivo* should be considered. In line with this is the study of Maier and colleques (1995) demonstrating that the onset of pro-inflammatory cytokine mRNA expression appears to be temporally associated with histological evidence of activation of microglial cells in the hippocampus.

The aforementioned results from *in vitro* studies shed some light on TMT action; however, a warning should be given toward drawing conclusions from cell culture systems data to understand all aspects of TMT neurotoxicity. There are some reasons for that: (1) the *in vivo* application of the organotin most likely results in a chain of events and it is difficult to study selective neurotoxic effects and attribute them to specific cell types and subcellular targets, (2) some of the effects may require chronic exposure of target sites, and (3) some of TMT neurotoxic actions may be the result of indirect effects on the brain.

PLASTIC PHENOMENA ASSOCIATED WITH TMT TREATMENT

Widespread degeneration induced by TMT may have far-reaching consequences in the function of the brain. Returning to the morphological level of analysis, in addition to the hippocampus, areas of the cortex related to the limbic system are also extensively damaged by TMT. For example, neuronal loss within the entorhinal cortex (EC) is especially prevalent in the cells of layer II and as a consequence the perforant pathway demonstrates substantial degeneration (Balaban et al. 1988). The anatomical findings have led to the hypothesis that the hippocampus is reinnervated by nonaffected afferents. The support for this hypothesis can be found in the studies of Brock and O'Callaghan (1987) who described the recovery of synapsin I and p38 (phosphoproteins associated with synaptic vesicles) following TMT exposure. These results were interpreted as indicating that TMT-produced loss of neurones is followed by reactive synaptogenesis. The anatomical source of growing sprouts was not provided by these studies but one source might be those hippocampal neurones that survive exposure to the toxin, another one – the septohippocampal system. While the first source has not been systemically investigated, several reports indicate TMT-induced regrowth of the cholinergic projection in the adult rat (Woodruff and Baisden 1990, Cannon et al. 1994).

Granule cells appear to be the major postsynaptic sites able to form new functional connections since they survive exposure to the toxin but lose their synaptic inputs due to destruction to the EC (that normally projects to the distal granule cell dendrites in the outer molecular layer) and to pyramidal cells in CA4/CA3 (that normally innervates the proximal dendrites of granule cells in the inner molecular

layer) (Witter 1993). Immunocytochemistry for microtubule-associated protein 2 (MAP-2), a dendritic marker (see Johnson and Jope 1992), revealed an increased staining throughout the ML (Koczyk 1994). These findings, most probably reflecting changes in dendritic branching pattern and corresponding with the time-course of axonal cholinergic sprouting into this layer reported by others (Woodruff and Baisden 1990, Cannon et al. 1994), indicate that granule cells are able to remodel their dendrites during denervation/reinnervation process.

Although the mechanisms underlying plastic responses remain unknown the involvement of trophic factors has been suggested to play a role at least in some experimental situations (see Crutcher 1987). Very recent observations of Koczyk et al. (1996) indicate that plastic neuronal changes evoked by TMT are accompanied by induction of trophic activity (nerve growth factor and high affinity receptor for NGF) of astroglial origin. Thus one may speculate that astrocytes produce and secrete to extracellular milieu an active form of NGF that supports changes in neuronal cytoarchitecture. Still remains an open question what is the functional significance of the plastic rearrangements? It has been proposed (see Butcher and Woolf 1989) that some pathological states may be promoted and/or accelerated by alterations in mechanisms regulating the expression of cytoskeletal proteins giving rise to stabilize an aberrant neuronal morphology followed by synaptic reorganization, altered neurotransmission, etc. Within this context, astrocytes would exacerbate the degenerative cascade. Although such a possibility seems attractive, the paucity of available data at both molecular and cellular levels does not allow investigators to draw an unequivocal conclusion.

CONCLUDING REMARKS

The spectrum of intra- and intercellular processes which are affected by TMT is probably much wider than described above. Hopefully, poisoning from TMT will never become a widespread health problem in man. However, the vulnerability of the hippocampus and other limbic structures, especially the entorhinal cortex, to chemical insult and its consequences including plastic phenomena and devastating effect on cognitive processes makes it imperative that basic research on brain function should be continued. The use of TMT should provide us with a better understanding of the mechanisms leading to cell death as well as those underlying the potency of the brain for adaptive processes.

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