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The organotin compounds trimethyltin (TMT) and triethyltin (TET) but not tributyltin (TBT) induce activation of microglia co-cultivated with astrocytes

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ABSTRACT

The organotin compounds trimethyltin (TMT), triethyltin (TET) and tributyltin (TBT) show different organotoxicities in vivo. While TMT and TET induce a strong neurotoxicity accompanied by microglial and astroglial activation, TBT rather effects the immune system. Previously, we have shown in an in vitro co-culture model that microglial cells can be activated by TMT in the presence of astrocytes.

In this study, we wanted to investigate (a) if the neurotoxic organotin compound TET can also activate microglial cells in vitro similar to TMT and (b) if differences between the neurotoxicants TMT and TET on the one side and TBT on the other exist concerning microglial activation. Therefor, purified microglial and astroglial cell cultures from neonatal rat brains were treated either alone or in co-cultures for 24 h with different concentrations of TMT, TET or TBT and the basal cytotoxicity and nitric oxide formation was determined. Furthermore, morphological changes of astrocytes were examined. Our results show that microglial activation can be increased in subcytolethal concentrations, but only in the presence of astrocytes and not in microglial cell cultures alone. This increase was induced by the neurotoxicants TMT and TET but not by TBT.

Taken together, the differing microglia activating effect of the organotin compounds may contribute to the differing neurotoxic potential of this group of chemicals in vivo. In addition, our results emphasize the need for co-culture systems when studying interactions between different cell types for toxicity assessment.

1. Introduction

Among the trialkyltin compounds TMT and TET, in contrast to TBT, show different organotoxicities. While TMT and TET are known to be very neurotoxic, TBT rather effects the immune system (Snoeij et al., 1987). O'Callaghan and Miller (1989) have shown that acute postnatal exposure to TMT and to a lesser extent to TET, but not to TBT, decreased brain weight, lowered hippocampal concentrations of the neuronal proteins synapsin I or p38 and that of the oligodendroglial protein MBP (myelin basic protein), and increased the astroglial specific protein GFAP (glial fibrillary acidic protein). These differing effects make this group of compounds an interesting tool for the investigation of neurotoxic mechanisms in vitro.

So far, studies on neurotoxicity in vitro have been mainly focused on neuronotoxicity, i.e. direct adverse effects of chemicals on neurons. Nevertheless, it is getting more and more obvious that non-neuronal cells in the nervous tissue play a very important role during neuropathological processes and, thus, have to be considered for the development of in vitro systems to study neurotoxicity.

Some groups (Karpiak and Eyer, 1999; Röhl et al., 2001) examined the cytotoxicity of TMT, TET and TBT on astroglial cell cultures and found that TMT was significantly less potent than TET and TBT, which does not correlate with their neurotoxic potential in vivo. O'Callaghan (1988) has shown for the neurotoxic organotin compounds that the neuronal damage is typically accompanied by glial cell activation (gliosis) and suggested to take the astroglia specific intermediate filament protein GFAP (glial fibrillary acidic protein), which is typically increased in reactive astrocytes (astrogliosis), as one biochemical marker of neurotoxicity in vivo. Nevertheless, aiming to establish an in vitro system to examine a possibly neurotoxic potential of a substance, we have shown previously that in primary brain cell cultures no GFAP increase could be induced by TMT, TET or TBT (Röhl et al., 2001) and that GFAP might be unsuitable as in vitro marker for neurotoxicity, because GFAP levels in untreated astroglial cultures are high and not constant (Röhl et al., 2003). Microglial cells also show typical morphological and functional changes during neuropathological processes in the brain. They become activated and release radical species and pro-inflammatory cytokines, possibly leading to neuronal damage as described in vivo for TMT (Maier et al., 1995; McCann et al.,

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1996; Bruccoleri et al., 1998). In contrast to the missing GFAP increase in astroglial cell cultures, an activation of microglial cells can be induced by TMT in vitro (Monnet-Tschudi et al., 1995; Maier et al., 1997; Viviani et al., 2001; Figiel and Dzwonek, 2007). Nevertheless, this effect seems to depend on interactions with other neural cells, as we have shown that the microglia was not activated by TMT in mono-cultures, but in co-cultures with astrocytes (Röhl and Sievers, 2005). Different co-culture models used during that study, including experiments with conditioned medium of TMT-treated astrocytes, could not reveal if this microglial–astroglial interactions depend on direct cell contact or on soluble factors, probably because of a weaker effect, which could not be detected with the used methods anymore.

The purpose of the present study was to compare the effect of TMT, TET and TBT on microglial activation in vitro and to answer the question, if this effect might correlate with the differing neurotoxic potential of these trialkytins observed in vivo.

To take the importance of glial cell interactions into account, we used microglia and astrocytes each in mono-culture as well as in co-culture systems with direct cell contact. The cells were treated for 24 h with different concentrations of one of the three organotin compounds together with a low concentration of lipopolysaccharides (LPS), which was added to sensitise the microglia to possibly activating stimuli. Then, the cytotoxicity and microglial activation were determined in the different cell culture systems and morphological changes of the astrocytes in mono-cultures were examined to infer if alterations of the astroglial cell morphology in response to the three trialkyltins differ corresponding to their microglia activating potency.

2. Materials and methods

2.1. Glial cell cultures

Astroglial cell cultures were prepared according to the method of McCarthy and DeVellis (1980) from neocortices of 2-day-old Wistar rats. This method allowed the preparation of nearly pure cultures of astrocytes with less than 0.5% microglial cells (Röhl et al., 2008). Astrocytes were seeded after 14 days of growth and purification in primary brain cell cultures at a density of 30,000 cells/cm^2 into 96-well microtiter plates for protein and nitric oxide measurements and the MTT assay, and into 24-well microtiter plates for phase contrast microscopy. The astroglial culture medium (DMEM (Gibco Invitrogen, no. 41965) supplemented with 10% (v/v) FBS (56 °C heat-inactivated, Gibco Invitrogen, no. 10270), 1% (v/v) penicillin (10,000 U/ml)/streptomycin (10 mg/ml)) was changed three times per week (310 µl/cm^2).

Microglia was prepared from mesencephali and neocortices of 2-day-old Wistar rats as described previously (Röhl and Sievers, 2005). Microglial cells harvested from supernatants of primary brain cell cultures were replated at a density of 100,000 cells/cm^2 into 96-well microtiter plates in astrocyte-conditioned medium made of astroglial culture medium to improve microglia attachment and survival. Culture medium was not changed until experiments were performed.

For microglial–astroglial co-cultures microglial cells were seeded at a density of 100,000 cells/cm^2 on top of 4-day-old subconfluent astroglial monolayers (seeding density 30,000 cells/cm^2) into 96-well microtiter plates in astroglial culture medium.

All cells were kept at 37 °C and 7.8% CO_2 until experiments were performed.

2.2. Organotin treatment

Seven-day-old confluent astroglial cultures, 4-day-old microglial cultures and 4-day-old microglial–astroglial co-cultures were treated for 24 h with different concentrations of TMT, TET or TBT, together with 0.02 µg/ml LPS (L-8275, Sigma) in culture medium. The test compounds were dissolved in DMSO and diluted with culture medium (final DMSO concentration 0.2%). The chosen test concentrations were 2–2000 µM (TMT), 0.1–50 µM (TET) and 0.02–5 µM (TBT). Wells were filled with 100 µl of organotin com-

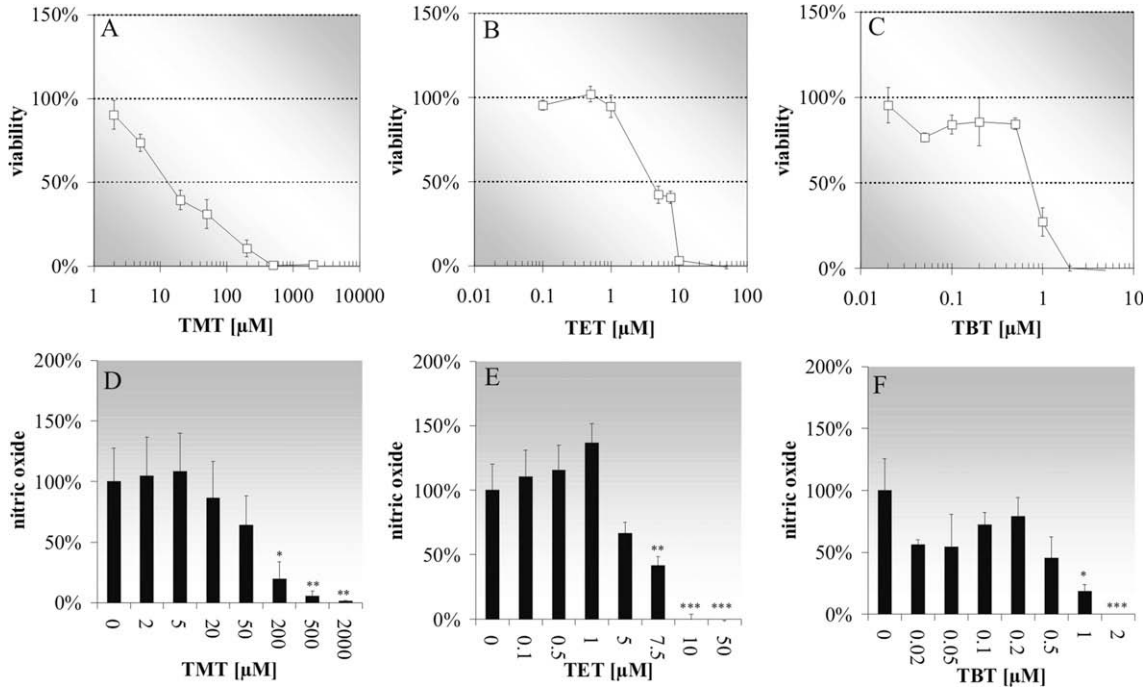

Fig. 1. Effect of TMT, TET and TBT on viability (MTT assay, A–C) and nitric oxide formation (Griess assay, D–F) of microglial cells in monolayer cultures. Three-day-old microglial cultures were treated for 24 h with different organotin compound concentrations and 0.02 µg/ml LPS. Each square or bar represents the mean ± SE of $n = 3$–5 independent experiments expressed as percentage of controls (no organotin compound, but LPS). D–F: $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ vs. control (repeated measures ANOVA followed by Tukey–Kramer multiple comparisons test).