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The environmental obesogen tributyltin chloride acts via peroxisome proliferator activated receptor gamma to induce adipogenesis in murine 3T3-L1 preadipocytes☆

Xia Li[a,1], John Ycaza[a,1], Bruce Blumberg[a,b,*]

[a] Department of Developmental and Cell Biology, 2011 Biological Sciences 3, University of California, Irvine, CA 92697-2300, United States
[b] Department of Pharmaceutical Sciences, University of California, Irvine, CA, United States

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ABSTRACT

Obesogens are chemicals that predispose exposed individuals to weight gain and obesity by increasing the number of fat cells, storage of fats into existing cells, altering metabolic rates, or disturbing the regulation of appetite and satiety. Tributyltin exposure causes differentiation of multipotent stromal stem cells (MSCs) into adipocytes; prenatal TBT exposure leads to epigenetic changes in the stem cell compartment that favor the production of adipocytes at the expense of bone, in vivo. While it is known that TBT acts through peroxisome proliferator activated receptor gamma to induce adipogenesis in MSCs, the data in 3T3-L1 preadipocytes are controversial. Here we show that TBT can activate the RXR–PPARγ heterodimer even in the presence of the PPARγ antagonist GW9662. We found that GW9662 has a 10-fold shorter half-life in cell culture than do PPARγ activators such as rosiglitazone (ROSI), accounting for previous observations that GW9662 did not inhibit TBT-mediated adipogenesis. When the culture conditions are adjusted to compensate for the short half-life of GW9662, we found that TBT induces adipogenesis, triglyceride storage and the expression of adipogenic marker genes in 3T3-L1 cells in a PPARγ-dependent manner. Our results are broadly applicable to the study of obesogen action and indicate that ligand stability is an important consideration in the design and interpretation of adipogenesis assays.

1. Introduction

The environmental obesogen model proposes that chemical exposure is a previously unappreciated risk factor for overweight and obesity [1]. Obesogens are functionally defined as chemicals, (dietary, endogenous, pharmaceutical, or xenobiotic), which, in combination with the more widely known and accepted factors of excess caloric input and reduced energy expenditure, predispose an exposed individual to subsequent weight gain and obesity (reviewed in [2,3–6]). Obesogens can act by increasing the number of adipocytes or stem cells committed to the adipocyte lineage, or by altering basal metabolic rate, shifting energy balance to favor the storage of calories and by altering the hormonal control of appetite and satiety (reviewed in [2,3–5,7]). An increasing number of obesogens have been identified in recent years and this field of study is expanding rapidly.

One of the more well-understood obesogens is the organotin, tributyltin (TBT). We and others have shown that TBT exposure leads to increased differentiation of pre-adipocytes in vitro [8,9], increased deposition of fat in vivo [8] and differentiation of multipotent stromal stem cells (MSCs) into adipocytes in vitro [10,11]. TBT and the related compound triphenyltin are high affinity agonists for two nuclear receptors that are important for adipogenesis: the peroxisome proliferator activated receptor gamma (PPARγ) and the 9-cis retinoic acid receptor (RXR) [8,9]. Prenatal exposure to TBT altered cell fate in the MSC compartment to favor the development of adipocytes at the expense of the bone lineage [10]. In accord with its molecular activity, we showed that TBT increased adipogenesis and adipogenic commitment in MSCs by activating PPARγ and that blocking PPARγ action with the potent and selective antagonist GW9662 strongly inhibited adipogenesis [10]. While it has not yet been demonstrated that TBT acts through PPARγ in the in vivo exposure model, it is clear that PPARγ activation is required for MSCs to enter the adipogenic pathway (reviewed in [12]).

However, in contrast to what is known about the role of PPARγ in MSCs, the situation in murine 3T3-L1 pre-adipocytes is less clear.

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* Corresponding author at: Department of Developmental and Cell Biology, 2011 Biological Sciences 3, University of California, Irvine, CA 92697-2300, United States. Tel.: +1 949 824 8573; fax: +1 949 824 4709.
E-mail address: Blumberg@uci.edu (B. Blumberg).

[1] Both the authors contributed equally to this work.

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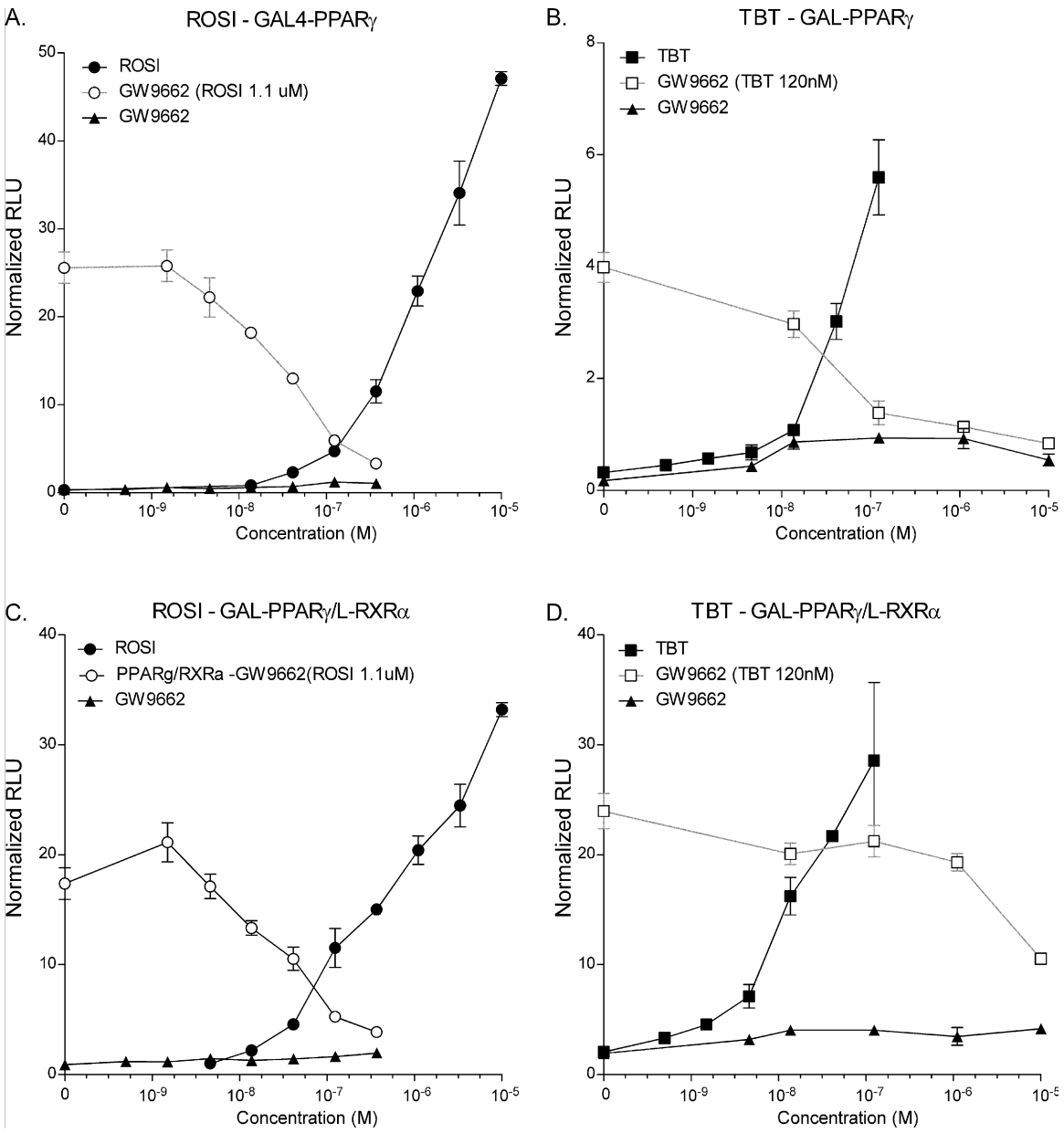

Fig. 1. The RXR–PPARγ heterodimer is permissive for RXR activation in the presence of GW9662. Cos-7 cells were transfected with the indicated receptor expression plasmids together with MH100x4-tk-luc reporter and CMX-β-galactosidase transfection control in 96-well plates and treated with a dilution series of the indicated test compounds or with a dilution series of GW9662 and a constant amount of ROSI (1.1 μM) or TBT (120 nM). (A and B) Activation of CMX-GAL-PPARγ by ROSI or TBT. (C and D) Activation of CMX-GAL-PPARγ + L-RXRα by ROSI or TBT. Data are expressed as relative light units normalized to β-galactosidase transfection control and represent the average ± SEM of triplicates. Experiments were performed at least 3 times.

At least one group has shown that GW9662 is unable to inhibit TBT-mediated adipogenesis in these cells and they concluded that adipogenesis in 3T3-L1 cells might not be dependent on PPARγ, or any other nuclear receptor for that matter [13]. Spiegelman and colleagues showed that PPARγ activity is required for adipogenesis in 3T3-L1 cells using the very low affinity PPARγ antagonist bisphenol A diglycidyl ether (BADGE) [14]. They subsequently demonstrated that while PPARγ itself was required (together with a functional AF2 activation domain), the ability of PPARγ to be activated by ligand appeared to be dispensable for adipogenesis; although, the presence of an endogenous PPARγ ligand could not be excluded [15]. Since 3T3-L1 cells are very commonly used and important model for adipocyte differentiation, we sought to understand these discrepancies and determine whether PPARγ activity was required for the induction of adipogenesis by TBT.

There are at least four possible reasons to explain the observation that TBT could cause adipogenesis in 3T3-L1 cells but that this induction could not be blocked by treatment with GW9662 [13]. The first and most obvious is that the process is not PPARγ mediated as has been suggested by other investigators [13]. We considered this possibility unlikely due to the well-established requirement for PPARγ in the adipogenesis of 3T3-L1 cells [14–16] and our results in MSCs [10]. A second possibility is that the RXR–PPARγ heterodimer is permissive for RXR activation even in the presence of a PPARγ antagonist such that pro-adipogenic genes normally targeted by this heterodimer are activated despite the antagonist. A third possibility is that the agonists and antagonists have different relative stabilities or persistence in culture; although, there are no data available on this point. Lastly, TBT might act through RXR homodimers to induce adipogenesis via RXR–PPARγ target genes