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Activation of brain estrogen receptors in mice lactating from mothers exposed to DDT

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Abstract

The insecticide dichlorodiphenyltrichloroethane (DDT) interferes with physiological endocrine processes modulating estrogens receptor activity. Most of the data describing the DDT mechanism of action have been collected in vitro or in reproductive tissues in vivo. Here we use a new transgenic mouse model to investigate the DDT effects on estrogens receptor activation in vivo in non-reproductive tissues. In particular, we demonstrate that DDT is able to activate estrogen receptors in the brain and the liver of adult mice after acute administration, and it is active in lactating mice when accumulated in the mother's milk. Furthermore, we demonstrate that the acute administration of DDT activates estrogen receptors with a different kinetics with respect to 17β-estradiol. Experiments with a breast cancer cell line engineered to express luciferase under the transcriptional control of activated estrogen receptors reveal that the microsomal metabolization of DDT is required for its full activity on estrogen receptors. Taken together these data lead to hypothesize that the delayed DDT time course on estrogen receptor activation in vivo might be due to a necessary step of metabolism of the compound.

Keywords: Hormones; Endocrine disruptors; Contaminants; Nervous system; Transgenic mouse; Metabolism

1. Introduction

In recent years several environmental contaminants, including pesticides and industrial chemicals have been found to disrupt endocrine functions in mammals. A large number of such compounds weakly bind to estrogen receptors (ERα and ERβ), evoking a biological response able to interfere with physiological endocrine processes. Dichlorodiphenyltrichloroethane (DDT) an insecticide widely used in agriculture, is a xenoestrogen that binds to ERα [5] and induces estrogenic effects in female rodents [18,35]. Although the majority of industrialized countries have banned the use of this pesticide for years, DDT and the metabolites still contaminate many natural places because of their long half-life, causing serious problems to animal species living there.

In wildlife the exposure to such compounds induces morphological, functional and behavioral anomalies associated with reproduction in birds, fishes, turtles and mammals. Epidemiological studies have shown that DDT and other endocrine disruptors are hazardous also for human health. It has been hypothesized that their accumulation in human population may be responsible for the decrease in the quantity and quality of human sperm during the last five decades of life, as well as increased incidence of testicular cancer, prostate cancer and cryptorchidism in males, and breast cancer and endometriosis in females [8]. Moreover, reports on the adverse effects of occupational exposure to DDT raised the suspicion of possible activities on the nervous system [33].

Owing to the lipophilic structure, DDT accumulates in tissues that contain a high rate of lipids such as neuronal cells. High concentrations of the metabolite 1,1-dichloro-2,2-bis (*p*-chlorophenyl)-ethylene (*p*,*p*′-DDE) were indeed found in the brain of stillborn infants in Georgia in the mid-1960s,

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when DDT was largely used in the United States [9]. Several studies have shown that the treatment with this toxic compound during early life causes neurobehavioral effects on mice [14,25], such as an increase of locomotor activity associated with a decrease in muscarinic cholinergic receptor density in the cerebral cortex [13]. Moreover, it was found that the exposure to DDT has also harmful effects on human brain development and seems to alter mental capabilities [12]. Because estrogens are known to play a fundamental role in the development and functionality of the central nervous system, it is very likely that the changes in cognitive function observed are mediated by the estrogens-like action of DDT.

To investigate the perturbing effects of DDT on the physiological activation of ERs, we used a transgenic model recently generated in our laboratory named estrogen-responsive elements (ERE)-Luc mouse [6]. With this model it is possible to detect the activity of the endogenous hormone [7] as well as exogenously administered estrogenic compounds [6,11]. In the present study we show that *p,p′*-DDT can activate ERs in non-reproductive organs such as brain and liver in adult and in suckling mice. The kinetics of the in vivo activation of ER by DDT is delayed with respect to 17β-estradiol. In addition, using a breast cancer cell line engineered to express a reporter of ER activity, we show that DDT metabolization by liver microsomes strongly increases its estrogenic activity. This allowed us to hypothesize that metabolization of the DDT is required to exert its estrogenic activity in vivo.

2. Materials and methods

2.1. Reagents

Unless otherwise specified reagents were purchased by Merck KgaA (Darmstadt, Germany).

2.2. Experimental animals

The procedures involving the animals and their care were conducted in accord with institutional guidelines, which comply with national and international laws and policies (National Institutes of Health, Guide for the Care and Use of Laboratory Animals, 1996, 7th ed., Washington, DC; National Academy Press, National Research Council Guide, www.nap.edu/readingroom/books/labrats). All the mice were kept in animal rooms maintained at a temperature of 23 °C, with natural light/dark cycles and with free access to food and water. ERE-Luc transgenic mice were generated as previously described [6] by oocytes microinjection. The construct used for transgenesis contained a luciferase reporter gene under transcriptional control of an estrogen-inducible promoter constituted by two estrogen-responsive elements (ERE) placed upstream of a thymidine kinase minimal promoter. Luciferase is virtually ubiquitously expressed in the ERE-Luc animals and can be induced by estrogen in target organs [6]. For the present experiments, we used heterozygous littermates obtained by mating our founders with C57BL/6 wild-type mice. Heterozygous transgenic male mice were screened by polymerase chain reaction analysis for the presence of the transgene. Heterozygous male mice (2 months old) were injected i.p., with 100 μl of 17β-estradiol (from Sigma, Steinheim, Germany),or *p,p′*-DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] (from Superchrom, Milan, Italy) at the needed concentration or with 100 μl of vehicle (vegetal oil) as control.

2.3. Luciferase enzymatic assay

At the time of death of the animals, by cervical dislocation, the tissues were dissected and immediately frozen on dry ice. Tissue extracts were prepared by homogenization in 500 μl of 100 mM K_2PO_4 lysis buffer (pH 7.8) containing 1 mM dithiothreitol, 4 mM EGTA, 4 mM EDTA, and 0.7 mM phenylmethylsulfonylfluoride, with a cycle of freezing-thawing and 30 min of microfuge centrifugation at maximum speed. Supernatants, containing luciferase, were collected, and protein concentration was determined by Bradford's assay [2]. Luciferase enzymatic activity was measured, as reported by de Wet et al. [10] in tissue extracts at a protein concentration of 1 mg/ml. The light intensity was measured with a luminometer (Murex Diagnostici, Rome, Italy) over 10 s and expressed as relative light units per microgram proteins.

2.4. Ligand-binding assays

The progesterone receptor (PR) concentration in the cytosols from liver was measured by the charcoal-dextran method, at a protein concentration between 1 and 2 mg/ml, and carried out by means of the multiple-saturation analysis (range of 3H-ORG2058: 0.75–12 nm) in the presence or absence of a 100-fold excess of cold progesterone as competitor. The values were plotted by the method of Scatchard [28]. Proteins in cytosol were determined by the method of Bradford [2].

2.5. Western blot analysis

Tissue extracts were prepared by homogenization in 200 μl of lysis buffer containing 420 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 20 mM Hepes pH 7.9, 20% glycerol, 0.1% Triton X-100, 5 mM β-mercaptoethanol, 0.1 mM PMSF followed by 30 min of microfuge centrifugation at maximum speed. Supernatants were collected and protein concentration was determined by Bradford's assay [2]. Equal amounts of proteins were loaded on a 10% SDS-PAGE and transferred onto nitrocellulose membrane. The filter was probed with monoclonal ERα antibody H222 diluted 0.1 μg/ml (kindly provided by Prof. G. Greene) and secondary antibody anti-rat conjugated with horseradish peroxidase (HRP) diluted 1:2000 (Chemicon Int., Inc., Temecula, CA) or with β-actin antibody 1:500 (Sigma, St. Louis,