

Alteration in Prolactin Secretion in Female Ovariectomized Rats by some Endocrine Disrupting Chemicals

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Citation

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Abstract

There is growing concern that many environmentally important chemicals may be capable of causing a variety of reproductive and endocrine disorders. Recent data seem to suggest that various xenobiotics have the potential to physiologically mimic important estrogen-regulated processes. It has also been shown that certain endocrine disrupting chemicals may have tumor-promoting potential via their ability to alter hormonal control. The purpose of this study is to examine the effects of several chemicals that have been implicated in endocrine disruption on the estrogen-mediated release of prolactin from the adult female rat pituitary gland. Ovariectomized female Sprague Dawley rats animals received DDT, methoxychlor, toxaphene, dieldrin, endosulfan, 17 β -estradiol (E-17), and 30 μ g/kg or the corn oil vehicle daily for seven days. Immediately following the exposures, blood samples were collected and plasma prolactin levels were measured by radioimmunoassay using [125I] rat prolactin as a tracer. Brain (TIDA) dopamine and DOPAC were also determined.

INTRODUCTION

Recent reports have suggested that dietary and environmental estrogens may play a role in the increased incidence of breast cancer, which has been documented over the last two decades (Safe1994). Much of the concern has involved the potential of environmental xenoestrogens to induce or promote carcinogenesis because of their ability to mimic estrogen. Studies have indicated that chlorinated organics have the effect of functioning directly and indirectly as xenoestrogens and hence, may increase the lifetime exposure to estrogen (Raloff 1994). Their potential contribution to the development of cancer and reproductive dysfunction in both humans and wildlife species has led to several reports in both the popular and scientific press that describe the perils of exposure to endocrine disrupting chemicals (Sugiura-Ogasawara 2005 et al., Iguchi and Sato 2000, Walker et al. 1980, Stirling and Shin 1990, Meites 1972, Davis et al. 1993, and Bradley et al. 1976).

One of the most important physiological functions of endogenous estrogens is regulation of the release of prolactin. Lactotrophs, which produce and secrete prolactin, constitute approximately 30% of the cells of the anterior pituitary. Mammalian prolactin exerts a wide range of physiological effects including mammary gland development and lactation. Prolactin synthesis is controlled principally by decreasing the rate of transcription of the

prolactin gene, which is predominantly controlled by the hypothalamus. A prolactin-inhibiting factor (PIH), dopamine, inhibits prolactin synthesis. When the pituitary stalk is severed or when the anterior pituitary is transplanted away from its hypothalamic connections, prolactin synthesis increases while synthesis of all other anterior pituitary hormones decreases. Lactotrophs are thus unique among anterior pituitary cells in being under major hypothalamic restraint. Estrogen acts directly on the pituitary gland to stimulate prolactin release, and also on the hypothalamus to decrease PIH activity by altering catecholamine concentrations.

Experimental evidence reveals that estrogenic chemicals and pharmaceuticals may affect estrogen production and metabolism and thus function as xenoestrogens (Steinmetz et al. 1997 and Meites 1972). Recent epidemiological studies have found that breast fat and serum lipids of women with breast cancer contain significantly elevated levels of some chlorinated organics as compared with cancer free control specimens. (Davis et al. 1993) and xenoestrogens may play a significant role in breast cancer worldwide (Raloff 1994).

The estrogenicity of chlorinated organics has been studied (Safe1994, Davis et al. 1993, and Soto et al. 1994). These compounds have the ability to stimulate estrogen dependent functions by various mechanisms. Since xenoestrogens may

have the effect of functioning in a manner similar to estrogen and since estrogen stimulates prolactin secretion, such compounds could be reasonably presumed to cause hyperprolactinemia. This would be measured as an increase in prolactin serum levels and or a change in catecholamine concentrations in TIDA neurons. This information could be used as an indicator or marker of estrogenic action and of disruption of endocrine function. Determination of serum prolactin levels may be a viable method of testing for estrogenicity. The hypothesis of this study is that since estrogen will increase serum prolactin levels, it follows that increased exposure to estrogenic chemicals may also enhance prolactin secretion.

MATERIALS AND METHODS

Animals, Chemicals, and Treatments: 35 (7 groups of 5) Sprague-Dawley (130g, Taconic Farms, Germantown, New York) were ovariectomized and acclimated for 2 weeks before treatment or use. The animals were housed in an air-conditioned room with lighting from 0600 to 1800 under conditions prescribed and approved by the IACUC. After the two week period the animals were randomly assigned to groups and dosed (table I.).

Figure 1

Table 1

Group 1	Control (vehicle corn oil) 1.6 ml/kg
Group 2	17 beta-Estradiol 30 µg/kg
Group 3	DDT 50 mg/kg
Group 4	Methoxychlor 200 mg/kg
Group 5	Toxaphene 10 mg/kg
Group 6	Dieldrin 10 mg/kg
Group 7	Endosulfan 10 mg/kg

Each of the chemicals were dispersed in corn oil and then subcutaneously injected daily over a 7-day period. Since these chemicals are lipophilic and dispersed in corn oil, SC injection allows for a slower sustained absorption and the injections can be spread out over the 7-day period. The dosage of the chemicals represents maximal amounts that would not be expected to produce acute lethal toxicity in most of the animals, but have been shown to induce estrogenic type responses. After 7 days, the animals were decapitated and trunk blood was collected into a heparinized tube (<20 units' heparin per ml of blood). Blood was centrifuged for 1000-x g for 10 min. to separate cells. The plasma was stored below -20 °C prior to analysis (Sternal et al. 1963).

Prolactin was measured by radioimmunoassay (Niswender et al. 1969 and Amersham 2003). The assay is based on the competition between unlabelled rPRL and a fixed quantity of

[¹²⁵ I]- labeled rPRL (Amersham Diagnostic Products Corp.) for a limited number of binding sites on a rPRL specific antibody. With fixed amounts of antibody and radioactive ligand, the amount of radioactive ligand bound by the antibody will be inversely proportional to the concentration of added non-radioactive ligand. The antibody bound rPRL is then reacted with the Amerlex-M second antibody reagent which contains a second antibody that is bound to magnetizable polymer particles. Separation of the antibody is effected by magnetic separation and decantation of the supernatant. Measurement of the radioactivity in the pellet allows the amount of labeled rPRL in the bound fraction to be calculated. Following the radioimmunoassay procedure, the radioactivity of each sample and standard was determined using a gamma scintillation counter. The concentration of unlabelled rPRL in the sample is then determined by interpolation from a standard curve.

TIDA neuronal activity can be estimated by measuring concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) and dopamine in the median eminence. The concentrations can be used a measure of dopamine metabolism. After decapitation, the rat brain was removed and frozen. Using a micropunch technique, the median eminence was dissected and sections of the median eminence were then digested with tissue buffer, citric acid and methanol. After centrifugation, the supernatant was collected for the catecholamine assays. Catecholamine and metabolite concentrations were assayed by HPLC with a reverse phase C₁₈ column following the methods outlined in Andrews' et al. Dopamine, and 3,4-dihydroxyphenylacetic acid (DOPAC) were identified on the basis of their peak retention times and quantified by comparison with external standards.

STATISTICS

The significance of differences among the groups was tested using the one way analysis of variance (ANOVA) followed by the LSD post hoc analysis to test for differences among the means of the groups. Values with P<0.05 were considered significant.

RESULTS AND DISCUSSION

Results of the rat PRL assay indicated that the groups administered estrogen, toxaphene, and methoxychlor, all had elevated PRL levels (p<0.05) as compared to the vehicle treated animals. The other chemicals tested, DDT, dieldrin, and endosulfan did not show any significant increase in PRL (Fig. 1). The median eminence DOPAC concentration was significantly (p< 0.05) reduced in the animals treated with

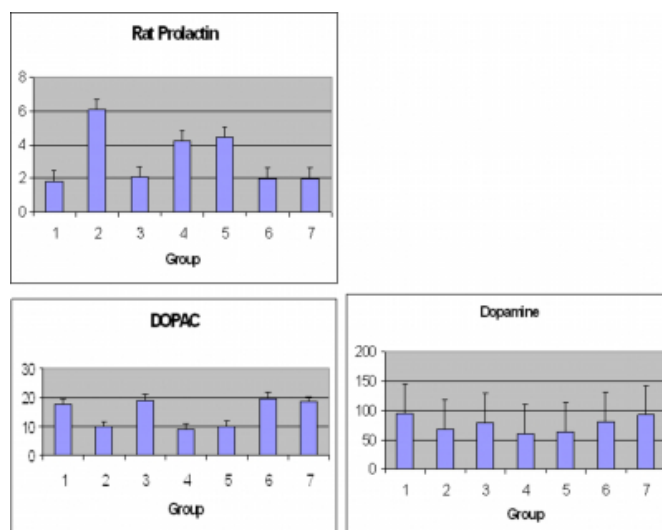
estrogen, toxephene and methoxychlor as compared with that of the controls. The dopamine concentration seemed to follow the same trend however, due to the high variation in dopamine concentrations within the groups, no statistically significant difference was observed for any of the groups. Changes in DOPAC were specific to the dopaminergic neurons and correlated with the observed increase in PRL (Fig. 1).

Since serum prolactin levels are sensitive to many drugs, especially those that are dopamine antagonists, it is quite surprising that there have been few attempts to measure serum prolactin levels following exposure to environmentally important xenobiotics that are capable of similar effects. The stated hypothesis of this study is that since estrogen will increase serum prolactin secretion, it follows that increased exposure to xenoestrogens may result in hyperprolactinemia. This study has shown that some environmental xenoestrogens are capable of inducing increases in circulating prolactin similar to those demonstrated with estrogen.

Estrogen alters the responsiveness of the anterior pituitary gland to the action of dopamine and hence to prolactin release. Three mechanisms may explain the estradiol-induced rise in prolactin: 1) direct stimulation on the anterior pituitary, 2) modulation of hypothalamic inhibiting factors such as dopamine, or 3) alteration in anterior pituitary responsiveness to prolactin. Much of the research to date supports the fact that the major control of prolactin release is via the effect of estrogen on dopamine release. This is supported by the fact that the anatomic link between the hypothalamus and the anterior pituitary must remain intact in order to observe a stimulatory effect from estrogen administration on prolactin release (Murai and Ben-Jonathan 1990).

Figure 2

Figure 1: Mean (\pm SEM), serum PRL Levels (ng/tube), TIDA DOPAC concentration (pg/ μ g protein), and Dopamine concentrations (pg/ μ g protein)



Treatment Groups

1. Corn Oil Vehicle
2. 17 beta-estradiol
3. DDT
4. Methoxychlor
5. Toxaphene
6. Dieldrin
7. Endosulfan

Estrogen has been shown to decrease the release of dopamine from the tuberoinfundibular neurons (Murai and Ben-Jonathan 1990), and dopamine has also been shown to inhibit prolactin release from the pituitary gland. Dopamine released from the tuberoinfundibular neuron is carried via the hypophyseal portal blood to the pituitary where it suppresses the release of prolactin. In the presence of estrogen, dopamine release is inhibited and the restraint is removed. This is further supported by the fact that selective activation of D2 receptors with dopamine agonists stimulate the tuberoinfundibular dopamine neurons and inhibit prolactin secretion from the anterior pituitary (Palmer and Palmer 1995). Clearly, decreases in dopamine activity are an important event controlling prolactin release from the pituitary following exposure to estrogen. The results of this study indicate that estradiol administration to

ovariectomized female Sprague-Dawley rats induces acute increases in the circulating levels of prolactin. The concentration of prolactin was significantly higher in rats exposed to estradiol when compared to those receiving the vehicle only. Furthermore, toxaphene and methoxychlor administration cause hyperprolactinemia while DDT, endosulfan, and dieldrin did not. One can speculate from these data that these chemicals differ in their affinities for the estrogen receptor and/or their mechanism of estrogenic actions.

The results of this study have shown that the xenoestrogens, methoxychlor and toxaphene, induce hyperprolactinemia, and mimic the inhibitory *in vivo* effects of 17 beta-estradiol on dopamine metabolism and turnover and therefore, and remove the inhibitory effects placed on the anterior pituitary lactotrophs and so stimulate prolactin release. In addition, methoxychlor and toxaphene may also act to decrease the number of D2 receptors on the anterior pituitary and further inhibit dopamine action in a manner similar to estrogen as shown by Pasqualini et al., 1990. In this regard, prolactin could be used as an indicator of estrogenic action and reproductive endocrine dysfunction. Another important consideration is that hormonal changes following exposure to xenoestrogens may also enhance mammary tumor development. Interestingly, of the chemicals used in this study only methoxychlor and toxaphene are suspected human carcinogens. A limitation of this study was that we exclusively used Sprague Dawley rats which are not as sensitive to estrogen as Fisher rats. Follow-up studies utilizing the Fisher rat may result in better responses to estrogenic chemicals.

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