Regulatory Peptides 185 (2013) 52–56

Contents lists available at ScienceDirect

Regulatory Peptides

journal homepage: www.elsevier.com/locate/regpep

Exposure to the endocrine disruptor nonylphenol alters structure and function of thyroid gland in rats

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ARTICLE INFO

Article history:
Received 8 January 2013
Received in revised form 17 April 2013
Accepted 14 June 2013
Available online 3 July 2013

Keywords:
Nonylphenol
Thyroid gland
Endocrine disruptors

ABSTRACT

Objective: Nonylphenol (NP) is an estrogenic-like compound which can induce vitellogenin synthesis in males and immature teleostean species. Known as an endocrine disruptor, it has been reported to affect endocrine glands; however, little is known about its effects on thyroid function. The present study aimed to evaluate whether exposure to NP alters the structure and function of the thyroid gland of rats and/or the underlying mechanisms.

Methods: Rats were gavaged with NP (40, 80 and 200 mg/kg/d) for 15 days. Serum levels of thyroid-stimulating hormone were determined by radioimmunoassay. Ultramicroscopic structure of follicular cells was examined by a transmission electron microscope. Histopathology was conducted with hematoxylin-eosin (HE) staining.

Results: We found that NP exposure induced a decrease in serum levels of free tetraiodothyronine (FT) 3 and FT4 while it induced an increase in serum levels of thyroid-stimulating hormone (TSH) in a dose-dependent manner. There was a negative correlation between different doses of NP with serum levels of FT3 and FT4 (FT4 $r = -0.932$; FT3 $r = -0.926$) and a positive correlation with serum levels of TSH ($r = 0.967$). Histological and morphometric study in the NP-exposed group revealed dilation of endoplasmic reticulum into cystic in thyroid follicular cells. Mitochondrion was damaged in the 80 and 200 mg/kg/d groups.

Conclusions: Exposure to NP may lead to thyroid dysfunction. It may be a potential contributor to thyroid disruption.

1. Introduction

There is increasing evidence from both epidemiology studies and animal models that specific endocrine-disrupting chemicals (EDCs) may influence reproductive disorders, dysplasia and the development or progression of cancers, such as breast cancer, ovarian cancer, testicular cancer and prostate cancer [1–3]. When wild-living animals were ascribed to EDCs, defects in sexual behavior and reproductive ability, as well as neurobehavioral and immune dysfunctions, appeared [4]. EDCs are compounds that alter the normal functioning of the endocrine system of both wildlife and humans. Growing evidence also suggests that EDCs may affect not only exposed individuals but also their children and subsequent generations. Altogether, EDCs pose a significant challenge to our industrialized society and to the health of humans and the environment.

Nonylphenol (NP) is a microbial degradation product of nonylphenol ethoxylates, the most prominent member of the alkylethoxylate surfactants [5]. NP is an estrogenic-like compound which can induce vitellogenin synthesis in males and immature teleostean species [6] NP has attracted attention as an estrogenic contaminant, and environmental pollution by NP has been extensive. NP has been found in wastewater and the sludge of sewage treatment works and river sediment, which is widely dispersed in the environment [7,8]. Some investigations have suggested that foods, including fish, meat and vegetables, and plastics used in food processing and packing are contaminated with NP [9,10], which is classified as a phenolic antioxidant based on its chemical activity and structure. Several studies have reported that NP can cause reproductive impairment and nerve, immune and cardiovascular system dysfunction [11–14]. Recently, Hao et al. reported that NP promotes adipocyte differentiation and induces obesity in mice [15]. As an antioxidant, it also induces and/or enhances carcinogenesis [16]. Furthermore, there is growing evidence that, in addition to the reproductive system, other endocrine systems such as the hypothalamus–pituitary–thyroid (HPT) axis may be targets of endocrine disruption. Because NP has been classified as an EDC and has weak ability to mimic estrogen (and in turn disrupts the natural balance of hormones in organisms) [17], we presumed that the estrogenic effect of NP may influence multiple endocrine systems. However, it is unclear whether NP affects thyroid function.

In the present study, we investigated the dose-dependent effect of NP on the function and structure of the thyroid gland in rats, which

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http://dx.doi.org/10.1016/j.regpep.2013.06.004

has always been used in models for exposure to EDCs. To this end, serum levels of thyroid-stimulating hormone were determined by radioimmunoassay. Ultramicroscopic structure of follicular cells was examined by a transmission electron microscope. Histopathology was conducted with hematoxylin-eosin (HE) staining. This was the first study to investigate the effects of NP on thyroid function. We believe that the data here reported will answer some of the questions regarding the toxicity of NP in animal endocrine physiopathology and contribute to the possibility of repeating and expanding this kind of research in the near future.

2. Materials and methods

2.1. Animal care and NP exposures

Forty female and male SD rats (4 wk old) of 140–160 g body weight were used in this study. They were obtained from Liaoning Medical College Laboratory Animal Center. The animals received a standard diet for rodents and were allowed free access to water. The animals were treated humanely and care was taken to ease suffering. We conducted all animal procedures according to the National Health and Medical Research Council guidelines and the animal experimentation ethics committee at Liaoning Medical College specifically approved this study. NP (Sinopharm Group Co. Ltd, Beijing, China) was dissolved in olive oil for all experiments. Rats were treated i.g. with NP (40, 80 or 200 mg/kg) per day or vehicle control [18–20]. All rats were treated for 15 days.

2.2. Tetraiodothyronine (FT) 3, FT4 and thyroid-stimulating hormone (TSH) measurements

At the end of the experimental period, blood was collected immediately for hormone analysis. Serum samples were obtained by centrifugation at 4 °C and stored in −70 °C until analysis. Levels of serum totals for FT3, FT4 and TSH were determined by radioimmunoassay using a commercial kit (Beijing North Institute of Biological Technology, Beijing, China).

2.3. Thyroid tissue preparation

Twenty-four hours after the last injection, all animals were anesthetized with phenobarbital sodium and, after intracardiac perfusion with the fixative solution (2% gluter-aldehyde in phosphate buffer), the thyroid glands were removed together with a portion of the adjoining trachea.

2.4. Morphological analysis

For the analysis of histology, dissected thyroid glands were fixed in 4% paraformaldehyde, embedded in paraffin and sliced at a thickness of 4 μm. The sections were stained using the HE method and investigated by using an inverted microscope, EUROMEX FE 2935 (ED Amhem, Netherlands), equipped with a CMEX 5000 camera system (4× and 40× magnification). The percentage of stratified epithelia was assessed independently by two individuals, including one consulting specialist in thyroid gland histopathology, and a mean percentage was generated. Ten readings per animal in each group were conducted. The following criteria for the diagnosis of thyroid follicular lesions were applied [21]: (1) Hypertrophy: decreased diameter of follicular lumens with increased height of follicular epithelium from cuboidal to tall columnar; (2) Hyperplasia: the above-mentioned changes with papillary infoldings of or stratification of follicular epithelium; (3) Adenoma: nonencapsulated well-demarcated proliferation of follicular epithelium with compression of adjacent follicles; (4) Carcinoma: solid or multiple layers of proliferation of follicular epithelium with cellular pleomorphism.

2.5. Electron microscopic study

Small fragments from the thyroid glands were rinsed in phosphate buffer (pH 7.4) fixed in 2% gluteraldehyde, postfixed in 1% osmium tetroxide and dehydrated. After embedding in ultrathin, sections were cut and stained with lead citrate and uranyl acetate. The grids were examined and photographed with an electron microscope [22].

3. Statistical analysis

Data are presented as mean ± standard deviation (SD). Comparison of FT3, FT4 and TSH between two groups was made with the q-test. Comparison among different groups was performed using an analysis of variance (ANOVA). Statistical significance was defined as $P < 0.05$. All statistical tests were carried out with the Statistical Package for the Social Sciences (SPSS) software (SPSS 11.5 Inc., Chicago, IL, USA).

4. Results

4.1. Changes of thyroid tissue morphology in rats

To study the effect of exposure to NP on the thyroid gland, we first investigated the possible changes of thyroid tissue structure. Histological examination was performed on the thyroid specimens processed by a routine HE staining procedure. As shown in Fig. 1, in the control group, the follicles are surrounded by a single layer of thyroid epithelial cells. After exposure to NP, thyroid follicular appeared in stratified epithelium. The percentage of stratified epithelium is approximately 16.95 ± 1.52%, 21.55 ± 3.46% and 27.64 ± 3.18%, respectively, in 40, 80 and 200 mg/kg groups, which appeared in a dose-dependent manner. We also found that the colloidal area percentage and connective tissue content were significantly increased in the NP-exposed group when compared to the control group. In the 200 mg/kg group, a trend of developing adenoma was evident.

4.2. Changes of thyroid ultrastructure in rats

Electron microscopic study of the thyroid in the control group showed eumorphism, nuclear membrane integrity, flat saclike rough endoplasmic reticulum (RER) in cytoplasm and rich chromatin and complex particles of retinal ribonucleoprotein rich in endoplasmic reticulum. However, there are many vesicular RERs in cells of NP-exposed rats. Damaged mitochondria were seen in 80 and 200 mg/kg NP-exposed rats, which showed swollen mitochondria, reduced mitochondrial crista and broken mitochondrial membrane (Fig. 2).

4.3. Changes of serum hormone levels in rats

We tested the possibility that structural modifications of the thyroid gland in rats exposed to NP could be related to functional changes in the thyroid. Table 1 shows the levels of FT3, FT4 and TSH in the serum. Animals exposed to NP for 15 days demonstrated decreased serum levels of FT3 and FT4, as well as increased TSH levels, when compared to the control group (Fig. 3, Table 1). There are significant differences in FT4, FT3 and TSH levels between the 40 mg/kg group and the 80 mg/kg group ($P < 0.05$).

In correlation tests, analyses of the correlation between doses of NP and levels of FT4, FT3 and TSH in serum, a statistically significant inverse correlation was found ($P < 0.05$) between levels of FT4 and FT3 and doses of NP, and a positive correlation was found ($P < 0.05$) between levels of TSH and doses of NP.