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The effect of *para*-nonylphenol on Japanese eel (*Anguilla japonica*) spermatogenesis in vitro

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Abstract

Endocrine disrupters have been recognized to interfere with endocrine systems that regulate reproduction, for example, by mimicking or inhibiting the action of endogenous sex steroid hormones including estradiol-17β (E2). In the present study, we examined the effect of an endocrine disrupter, *para*-nonylphenol (*p*-NP) on spermatogenesis, and compared it with the action of E2, using an eel testicular organ culture system. *p*-NP alone stimulated early spermatogonial renewal in the same manner as E2. Neither induced further progress in spermatogenesis. In the presence of 11-ketotestosterone (11-KT), the major androgen in teleosts, *p*-NP did not prevent the 11-KT-induced progress in spermatogenesis. However, this treatment enlarged the Sertoli cells. Electron microscopic observation revealed that enlarged Sertoli cells contained well-developed organelles. Moreover, the proportion of germ cells appeared to have decreased as a result of Sertoli cell hypertrophy. These results clearly show that *p*-NP has an effect on Sertoli cells in the presence of an androgen (11-KT), potentially disturbing 11-KT-induced spermatogenesis.

Keywords: Nonylphenol; Japanese eel; Spermatogenesis; Endocrine disruption; 11-Ketotestosterone; Testis; Sertoli cells; Estradiol-17β

1. Introduction

Anthropogenic chemicals are present in the environment as pollutants and interact with the endocrine systems of animals, including fish. Many of these chemicals interfere with estrogen receptor-mediated physiological responses. Such compounds are called endocrine disrupters and either evoke estrogenic responses by mimicking, or by inhibiting, the action of endogenous estrogen such as estradiol-17β (E2).

The testis is a target organ for endocrine disrupters, which seems to cause reproductive dysfunction such as a decrease in the number of spermatozoa (Carlsen et al., 1992; Irvine et al., 1996; Van Waeleghem et al., 1996).

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It is possible that endocrine disrupters affect spermatogenesis through estrogen receptors (ER). In previous research (Miura et al., 1999), we examined the function of female hormone "estrogen", E2, on spermatogenesis in Japanese eel. E2 was present in the blood and its receptors were expressed in the Sertoli and interstitial cells of testis during the complete spermatogenic process. In vivo and in vitro experiment showed that E2 promoted early spermatogonial renewal. This stimulatory effect was suppressed by tamoxifen, an antagonist of estrogen action in in vivo experiments. This study demonstrated that E2 has a potency to stimulate the proliferation of undifferentiated early spermatogonia. Further studies identified a growth factor as mediator of the E2-induced proliferation of undifferentiated spermatogonia (Miura et al., 2003).

In the present study, the effect on spermatogenesis of one estrogenic endocrine disruptor, *para*-nonylphenol (*p*-NP) was examined, in comparison with the action of E2. Nonylphenol etholxylates (NPE) are widely used as surfactants in industries. NPE is liberated by degradation of ethoxylated nonylphenol derivatives in natural waters, and the original compounds disappear rather rapidly from the environments. Nevertheless, some of NPs produced in the decomposing process are relatively persistent as the intermediates formed, such as short-chained NPE and, especially, free nonylphenol (Ahel et al., 1994). Consequently, free nonylphenol will presumably be widely distributed in surface waters and it has been shown to accumulate in various aquatic organisms (Ekelund et al., 1990; Ekelund et al., 1993). In flounder, NP acts on the male reproductive tissue and induces the formation of primary oocytes in testis tissue (Hashimoto et al., 2000). In addition, exposure to NP of male rats decreased testis size, seminiferous tubule diameter, lumen diameter and epithelium height compared to the control group (Lee, 1998; Chapin et al., 1999; De Jager et al., 1999a,b; Lee et al., 1999; Laws et al., 2000). There are a number of reports indicating that NP induced the synthesis of vitellogenin (VTG) with a significant reduction in the gonadosomatic index in male fish in in vitro and in vivo experiments (Sumpter and Jobling, 1995; Jobling et al., 1996; Korsgaard and Pedersen, 1998; Christensen et al., 1999).

To study the various effects of *p*-NP on spermatogenesis, Japanese eel (*Anguilla japonica*) was chosen as experimental model. In Japanese eel, spermatogenesis can be induced by injecting human chorionic gonadotropin (hCG) in vivo, or by adding hCG or 11-ketotestosterone (11-KT), the major androgen in fish, to testis organ culture or germ-somatic cells co-culture systems (Miura et al., 1991a,b,c; Miura et al., 1996). Employing these experimental systems, it is possible to study direct effects of endocrine disrupters on spermatogenesis.

2. Materials and methods

2.1. Animals

Cultivated male eel, *A. japonica*, (180–200 g of body weight) were purchased from a commercial eel supplier, and kept in circulating fresh-water tanks with a capacity of 500 l at 23 °C.

2.2. Organ culture techniques

Organ culture was carried out on an agarose substrate, which was a method for improving the floating method as described previously (Miura et al., 1991a,b,c). Briefly, a nitrocellulose membrane lies on a 1% agarose (Sigma) cylinder soaked with the culture medium; cylinders are set in the wells of 24-well culture dishes (Iwaki) containing 1 ml of culture medium. Fresh testes were removed, cut into small pieces (1 mm × 1 mm × 0.5 mm), and placed on a peace of nitrocellulose membrane (0.5 cm^2) on gel cylinders. They were then cultured in Leibovitz L-15 culture medium for eel (Miura et al., 1991b) with or without 10 ng/ml of 11-KT and 1 pg/ml to 100 μg/ml of *p*-NP (Wako Pure Chemical Industries, Ltd.) for 15 or 30 days in humidified air at 20 °C. In addition, testes were cultured with 100 pg/ml of E2 as a positive control. Controls were taken at day 0 (initial control, IC), at days 15 and 30 to control for possible base-line changes during the incubation period for each condition, six to eight replicates were incubated. The basal culture medium consisted of Leibovitz L-15 medium supplemented with 1.7 mM proline, 0.1 mM asparatic acid, 0.1 mM glutamic acid, 0.5% bovine serum albumin fraction V (Sigma), 1 μg/ml bovine insulin, and 10 mM Hepes, and adjusted to pH 7.4. The medium was changed after every 7 days. After the culture, testicular fragments were collected for light and electron microscopically observations.