4-Nitro-3-phenylphenol has both androgenic and anti-androgenic-like effects in rats

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Abstract. To investigate the effect of endocrine disruption of 4-nitro-3-phenylphenol (PNMPP) on immature male Wistar-Imamichi rats, the rat pituitary was exposed to PNMPP (10–5–10–9 M) for 24 h with or without gonadotropin-releasing hormone (GnRH) in experiment I. In addition, the Leydig cells (10–5–10–9 M) were exposed to PNMPP for 24 h with or without human chronic gonadotropin (hCG) in experiment II. Our results showed that the PNMPP at 10–5–10–7 M suppressed follicle-stimulating hormone (FSH) and luteinizing hormone (LH) productions from GnRH-stimulated pituitary cells. At the same time, PNMPP 10–5–10–7 M induced an increase in testosterone production from the Leydig cells treated with or without hCG. Based on our results, it can be concluded that PNMPP might have both androgen agonist action by decreasing FSH and LH production in the pituitary and anti-androgenic action by increasing testosterone production in the Leydig cell.

Key words: 4-Nitro-3-phenylphenol, Gonadotropins, Leydig cell, Pituitary, Testosterone

Materials and Methods

Chemicals

4-Nitro-3-phenylphenol (PNMPP), as shown in Fig. 1, was synthesized by the method described previously [2].

Animals

Immature male Wistar-Imamichi rats at 28 days of age were purchased from the Imamichi Institute for Animal Reproduction, Ibaraki, Japan. They were maintained under conditions of controlled lighting (14 h: light 10 h dark, lights on 0500 h), temperature (22 ± 2 C), and humidity (50 ± 5%). Food (CE-2 commercial diet; Clea Japan, Tokyo, Japan) and water were available ad libitum. All procedures were carried out in accordance with guidelines established by the Tokyo University of Agriculture and Technology, for use of laboratory animals.

Experimental procedure

The rats were decapitated, and the anterior pituitary gland and Leydig cells were removed immediately.

Experiment I: Effect of PNMPP on hormone secretion from the anterior pituitary

The anterior pituitaries were placed in cold DMEM medium containing 10 g/l M5M, 6 g/l HEPES, 10% NaHCO3, and 10 ml/l
cells were exposed to PNMPP (10^{-9}–10^{-5} M) dissolved in media. At the Leydig cells Experiment II: Effect of PNMPP on hormone secretion from 95% air and 5% CO_2. Then the culture media were changed, and the for 78 h in 96-well culture plates at 37 C under an atmosphere of ON, Canada). The pituitary suspension was cultured and incubated 100 U/ml penicillin and 100 µg/ml streptomycin (Invitrogen, Burlington, free serum; Wako Pure Chemical Industries, Osaka, Japan), 100 U/ml penicillin and 100 µg/ml streptomycin (Invitrogen, Burlington, ON, Canada). The pituitary suspension was cultured and incubated for 78 h in 96-well culture plates at 37 C under an atmosphere of 95% air and 5% CO_2. Then the culture media were changed, and the cells were exposed to PNMPP (10^{-9}–10^{-5} M) dissolved in media. At 24 h after exposure to PNMPP, the cells were stimulated with and without 0.1 IU/ml human chronic gonadotropin (hCG) dissolved in media. After 4 h of hCG stimulation, the medium was collected for testosterone assay.

Hormonal assays

FSH and LH concentrations were measured using an NIDDK radioimmunoassay (RIA) kits (Torrance, CA, USA) for rat FSH and LH. The iodinated preparations were rat FSH-I-5 and LH-I-5. The antisera used were anti-rat FSH-S-11 and anti-rat LH-S-11. Results were expressed as rat FSH RP-2 and rat LH RP-3. The intra- and interassay coefficients of variations were 4.8 and 11.4% for FSH and 5.4 and 6.9% for LH, respectively.

Testosterone concentration was measured using a double-antibody RIA system with ^{125}I-labeled radioligands as described previously [15]. Antisera against testosterone (GDN 250), provided by Dr GD Niswender (Colorado State University, Fort Collins, CO, USA), were used. The intra- and interassay coefficients of variations were 5.9 and 5.8%.

Statistical analysis

The data were expressed as means ± SE. One-way analysis of variance (ANOVA) was used to compare means among groups. Post hoc multiple comparison analyses were performed with the Least Significant Difference (LSD) test when the F ratio for the ANOVA was significant at P < 0.05.

Results

Effect of PNMPP on hormone production from the pituitary

As shown in Fig. 2, PNMPP treatment could not increase the concentrations of FSH and LH secreted from the pituitary cells without GnRH stimulation. Conversely, 10^{-5}–10^{-7} M of PNMPP could increase the FSH and LH concentration when the cells were stimulated with GnRH. On the other hand, 10^{-5}–10^{-9} M of PNMPP could not increase FSH and LH concentrations, although the cells were stimulated with GnRH.

Effect of PNMPP on hormone production from Leydig cell culture

Testosterone concentrations were significantly increased, showing an inverted U shape, in cultures of Leydig cells stimulated with and without hCG when the cells were treated with PNMPP (Fig. 3).

Discussion

In the present study, PNMPP (10^{-5}–10^{-7} M) reduced GnRH-stimulated FSH and LH secretions from anterior pituitary cells, but did not have any effect on the pituitary cells without GnRH stimulation. This result indicated that PNMPP played a role in decreasing FSH and LH secretion via GnRH stimulation. As we known, AR is found in the pituitary and affected by androgen administration and castration [16–20]. Androgen also has a role in control of GnRH released from the hypothalamus, as shown by the effect of testosterone treatment on reducing GnRH mRNA [17] and GnRH release [18]. In in vitro
studies, androgen was found to suppress pituitary responsiveness to a hypothalamic extract and changed FSH and LH release from the anterior pituitary [20, 21]. Androgen acts by binding at receptor sites and then has a negative feedback action that decreases FSH and LH secretions by slowing the GnRH pulse generator and suppressing FSH and LH syntheses in the pituitary [22].

A single treatment of 3-methyl-4-nitrophenol (4-nitro-m-cresol; PNMC), which was extracted from DEPs, suppressed the plasma LH concentration in Japanese quails [6]. PNMC increased the plasma testosterone concentration and decreased the plasma FSH and LH concentrations, indicating that it acts on the hypothalamus-pituitary axis in adult male rats [23] and immature male rats [24].

Our previous study found that the chemical structure of PNMPP comprises a benzene ring, which is similar to steroid hormones including estrogen and androgen [1]. Hence, in the study of a pituitary cell culture containing GnRH, we might assume that PNMPP acts as androgen and reduce the effect of GnRH action on the secretions of FSH and LH, which would be the same as the effect of PNMC found in previous papers [1, 23, 24].

In the present study, PNMPP induced high secretion of testosterone in the Leydig cells cultured with and without hCG when compared with the control. From previous in vitro studies, DEPs have been reported to slightly increase the gene expression of the steroidogenic acute regulatory (StAR) protein in mouse Leydig cells [25]. Exposure to nanoparticle-rich diesel exhaust (NR-DE) enhanced cholesterol synthesis and increased the expression of gene that regulate steroid synthesis along with the testosterone concentration in testicular culture [26]. Consistent with the study in vitro, exposures to NR-DE for 1 or 2 months significantly increased StAR and cytochrome P450 side-chain cleavage (P450scc) mRNA and their protein expressions and increased the testosterone concentration in male rats and mice [26, 27]. Either NR-DE or DEPs have a direct effect on testosterone production by increasing mRNA expression and genes associated with testosterone cholesterol synthesis in Leydig cells [23, 27, 28]. Accordingly, we assume that PNMPP may have a direct effect on increasing the testosterone concentration in Leydig cells.

Furthermore, addition of procymidone, an anti-androgenic substance, to a Leydig cell culture stimulated with hCG increased testosterone production by elevating several steroidogenic enzymes including StAR, P450scc and cytochrome P450c17α (P450c17) [29]. Flutamide, an androgen receptor antagonist, also enhanced StAR mRNA expression from Leydig cells of adult rats treated with hCG [30]. Furthermore, it was shown previous that anti-androgen caused hypergonadotropic activation of testicular steroidogenesis [2]. PNMPP has been reported to inhibit DHT action by binding to the androgen receptor in a recombinant yeast screen assay [2]. It can be assumed that PNMPP had an anti-androgenic effect on testosterone production in the Leydig cells.

In summary, the present study clearly demonstrated that PNMPP had androgen agonist action by suppressing the effect of GnRH and then decreasing the FSH and LH concentrations in the pituitary cell culture. In addition, PNMPP had an anti-androgenic effect on testosterone production in Leydig cell culture.

Acknowledgments

We are grateful to the Rat Pituitary Hormone Distribution Program, NIDDK, NIH, Bethesda, MD, USA, for providing RIA materials and Dr GD Niswender, Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO, USA, for providing antisera to testosterone (GDN 250). This study was supported by a grant in Aid for Scientific Research (C-26340037) from the Japan Society for the Promotion of Sciences. This work was supported by a Grants-in-Aid for Scientific Re-
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search (the 21st Century Center of Excellence Program, E-I) from the Ministry of Education Culture, Sports, Science and Technology of Japan (B18310044) and the Japan Society for the Promotion of Science (P05480).

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