Effects and Mechanisms of Nonylphenol on Corticosterone Release in Rat Zona Fasciculata-Reticularis Cells

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Alkylphenol ethoxylate, consisting of ~80% nonylphenol ethoxylate (NPEO), is a major group of nonionic surfactant. The primary degradation product of NPEO, nonylphenol (NP), interferes with reproduction, induces cell death in gonads, and leads to changes in other reproductive parameters. With such apparent stress, NP is believed to induce stress response mechanism, i.e., adrenal cortical hormone. However, the effects and action mechanisms of NP on rat adrenal zona fasciculata-reticularis (ZFR) cells are still unclear. This study explored the effects of NP on corticosterone release. ZFR cells were incubated with NP in the presence or absence of adrenocorticotropin (ACTH), 8-bromo-cyclic 3’,5’-adenosine monophosphate (8-Br-cAMP), forskolin (FSK), 25-hydroxyl cholesterol (25-OH-cholesterol), pregnenolone, progesterone, or deoxycorticosterone at 37°C for 1 h. The concentrations of corticosterone or pregnenolone in the spent media were measured by radioimmunoassay. The expressions of Leydig cell steroidogenic acute regulatory (StAR) protein, cytochrome P450 side-chain cleavage (P450scc) protein, and 11β-hydroxylase in the cells were measured by Western blot. The data demonstrated that (1) NP stimulated corticosterone release induced by ACTH, 8-Br-cAMP, FSK, 25-OH-cholesterol, pregnenolone, progesterone, or deoxycorticosterone; (2) NP significantly increased pregnenolone release in the control, 25-OH-cholesterol, triolostane, and 25-OH-cholesterol + triolostane groups; (3) NP-stimulated corticosterone release was estrogen receptor dependent, but mediated by nitric oxide and p38 mitogen-activated protein kinase pathway independent; and (4) NP did not affect StAR, 11β-hydroxylase, or P450scc protein expression. These results suggest that NP acts directly on rat ZFR cells to stimulate corticosterone release and that the stimulation mechanism of NP mediates through post-cAMP corticosterone manufacture enzymes, i.e., P450scc and 11β-hydroxylase.

Key Words: cAMP; NP; P450scc; 11β-hydroxylase; zona fasciculata-reticularis cells.

Nonionic surfactants possess specific physicochemical characteristics, including relative ionic insensitivity and sorptive behavior, which lead to their extensive applications in industry, processing technology, and detergents. Alkylphenol ethoxylate (APEO), consisting of ~80% nonylphenol ethoxylate (NPEO), is a major group of nonionic surfactants (de Voogt et al., 1997; Hawrelak et al., 1999). The primary degradation product of NPEO, nonylphenol (NP), has been documented in aquatic environments and sediment, where the concentration has approached milligrams per liter and milligrams per kilogram levels, respectively (Mark et al., 2001; Naylor et al., 1992; Shao et al., 2005). NP has weak estrogenic activity (Schwaiger et al., 2002; Van den Belt et al., 2004) and can interfere with reproduction in fish, reptiles, and mammals; induce cell death in gonads; and lead to changes in other reproductive parameters (Cardinali et al., 2004; Nagao et al., 2001; Weber et al., 2002; Wu et al., 2010a,b). Adrenal gland plays a role in the body response mechanism to stress and produces and secretes cortisol or corticosterone (in rodents). The knowledge of NP effect on corticosterone secretion is needed especially in public health area.

Nitric oxide (NO), synthesized from l-arginine by the enzyme NO synthase, is a small, gaseous, and reactive molecule that is involved in the regulation of a wide range of biological functions as an intercellular and intracellular signal. Increasing evidence suggests that NO participates in endocrine modulation, as it is implicated in the control of the hypothalamo-pituitary axis (Ceccatelli et al., 1993; Duvilansky et al., 1995; Rettori et al., 1993) and in the activity of pancreatic-β-islets (Schmidt et al., 1992). In particular, several reports suggest that NO is involved in the regulation of steroid biosynthesis. It has been shown that NO inhibits steroidogenesis in granulosa-luteal cells (Dave et al., 1997; Van Voorhis et al., 1994) and human chorionic gonadotropin-induced steroidogenesis in both MA-10 and rat Leydig cells (Del Punta et al., 1996). Yoshitake et al. (2008) proposed that endocrine-disrupting chemicals (bisphenol A [BPA], alkylphenols p-nonylphenol, p-n-octylphenol [OP], chlorinated phenols 2,4-dichlorophenol [DCP], and pentachlorophenol...
[PCP]) suppress NO production and nuclear factor kappa-light-chain-enhancer of activated B cells activation in lipopolysaccharide-stimulated macrophages through estrogen receptor (ER)-dependent (BPA, NP, and OP) and -independent (PCP and DCP) pathways. Cymering et al. (1998, 1999, 2002) proposed that NO may be a negative modulator of adrenal zona fasciculata or cell line Y1 steroidogenesis. Natarajan et al. (1997) proposed that NO can inhibit angiotensin II and adrenocorticotropic (ACTH)-induced aldosterone synthesis in rat and human adrenal glomerulosa cells. Mitogen-activated protein kinase (MAPK) signal transduction pathways are well-characterized signal networks. There are three major groups of MAPKs: extracellular signal-regulated kinase (ERK) 1/2, p38 (α/β/γ/δ), and c-Jun N-terminal kinase (JNK1/2/3) (Kyriakis and Avruch, 2001; Raman et al., 2007; Widmann et al., 1999). The JNK and p38 pathways are thought to be more responsive to environmental and physiochemical stresses. Abidi et al. (2008) proposed that oxidant-induced inhibition of adrenal steroidogenesis requires the participation of p38 MAPK. Aravindakshan and Cyr (2005) proposed that NP inhibits gap-junction intercellular communication between Sertoli cells and is modulated via an ER-independent mechanism and through inhibition of the p38 MAPK pathway. However, the knowledge on the direct effect of NP on steroidogenesis in rat zona fasciculata-recticularis (ZFR) cells and the involvement of an NO modulator or the p38 MAPK pathway is limited.

In the present study, rat ZFR cells were employed to investigate the direct effects and mechanisms of NP on corticosterone release. We evaluated the effect of NP on the steroidogenesis of ZFR cells, function of the cytochrome P450 side-chain cleavage (P450scc) and 11β-hydroxylase enzymes, expression of the steroidogenic acute regulatory (STAR), 11β-hydroxylase, or P450scc protein and NO modulator, p38 MAPK pathway, and ER dependent.

**MATERIALS AND METHODS**

**Animals.** Male Sprague-Dawley rats, weighing 300–350 g (2 months old), were provided by National Yang-Ming University and were housed in a temperature-controlled room (22 ± 1°C) with photoperiods of 14 h (light):10 h (dark). The light was on at 6:00 AM, and food and water were provided ad libitum.

The animal protocols were approved by the Institutional Animal Care and Use Committee of the National Yang-Ming University. All the animals received human care in compliance with the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals, published by the National Science Council, Taiwan, Republic of China.

**Reagents.** NP (Fluka, Buchs, Switzerland) was dissolved in methanol at 4.25M to produce a stock solution. The ER antagonist, ICI182780 (Tocris, Ellisville, MO), was dissolved in ethanol at 10mM to produce a stock solution. ACTH, bovine serum albumin (BSA), 8-bromo-cyclic 3',5'-adenosine monophosphate (8-Br-cAMP), collagenase, deoxycorticosterone, glucose, forskolin (FSK), Hank’s balanced salt solution, 25-hydroxyl cholesterol (25-OH-cholesterol), N-2-hydroxyethylpiperazine-N’-2-ethane sulfonic acid, 3-isobutyl-1-methyl-xanthine (IBMX, phosphodiesterase inhibitor), PMSF, pregnenolone, progesterone, SB203580, and sodium nitroprusside (SNP) were purchased from Sigma Chemical Co. (St Louis, MO). The above-mentioned steroids were dissolved in ethanol as stock solutions. The stock solutions were diluted in KRBGA medium (Krebs-Ringer bicarbonate buffer with 3.6 mmol K+/l, 11.1 mmol glucose/l, and 0.2% BSA) when used for challenging the cells. We observed that dilute ethanol or methanol did not kill the cells (data not shown). SDS and bromophenol blue were purchased from Research Organics Inc. (Cleveland, OH). Proteinase inhibitor cocktail tablets were purchased from Boehringer Mannheim (Mannheim, Germany). Tristolone (4,5-epoxy-17-hydroxy-3-oxoandrostan-2-carbonitrile), an inhibitor of 3β-hydroxysteroid dehydrogenase (3β-HSD) was provided by Sanofi-Synthelabo, Inc. (Malvern, PA). [1H]-corticosterone and [3H]-pregnenolone were obtained from Amershams Life Science Limited (Buckinghamshire, UK). The anti-pregnenolone antisemur was purchased from Biogenesis (Pool, England, UK), and anti-11β-hydroxylase antibody was obtained from Abcam (Cambridge, UK). The anti-P450scc antibody and anti-STAR antibody were kindly provided by Dr Bon-Chu Chung (Hu et al., 1991) and Dr D. M. Stocco (Lin et al., 1998), respectively. The peroxidase-conjugated IgG fraction to mouse IgG and peroxidase-conjugated IgG fraction to rabbit IgG were purchased from ICN Pharmaceuticals, Inc. (Aurora, OH).

**Preparation of ZFR cells for cell culture.** Rat adrenal glands were excised and then kept in an ice-cold 0.9% (wt/vol) NaCl solution. The adipose tissues were removed, and the encapsulated glands were separated by forceps into capsule (mainly zona glomerulosa) and inner zone (mainly ZFR) fractions. The inner zone fractions of 10–20 adrenals were pooled into one dispersion, and the ZFR cells were prepared as our previous (Lo et al., 1998). The cells (5 × 10⁴ cells/ml) were pre-incubated with KRBGA medium for 1 h at 37°C in a shaker bath (50 cycles/min) aerated with 95% O₂ and 5% CO₂. The supernatant was decanted after centrifugation at 200 × g for 10 min. Finally, the cells were resuspended in fresh incubation medium for 1 h. After incubation and centrifugation, the medium was stored at −20°C for corticosterone or pregnenolone radioimmunoassay (RIA), and the cells were used for Western blot.

To study the effect of NP on NO, p38 MAPK, or ER, the ZFR cells were primed with SNP (NO generator; Moncada et al., 1991), SB203580 (an inhibitor of p38 MAPK; Cueva et al., 1995), or ICI182780 (an antagonist of ER), for 30 min and then further incubated with ACTH combined with NP.

**Western blot analysis.** After incubation with or without the appropriate stimulant, ZFR cells were washed twice with ice-cold saline and then harvested in 50 μl of cell lysis buffer (1.5% Na-lauroylsarcosine, 2.5mM Tris-base, 1mM EDTA, 0.68% PMSF containing 2% proteinase inhibitors; pH 7.8). The cell lysates were centrifuged for 10 min at 13,000 × g. The supernatant was assayed for protein content (Bradford, 1976) and subjected to Western blot analysis (Kau et al., 1999a; Lo et al., 2000) to detect STAR, 11β-hydroxylase P450scc, or β-actin. Samples containing equal amounts of protein were separated by 10% acrylamide sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The relevant proteins were detected on blots using their specific antibodies and were visualized by chemiluminescence (ECL Western blotting detection reagents; Amersham Pharmacia Biotech, Buckinghamshire, UK). The chemiluminescence signal on the membranes was scanned by Luminescent Image Analyzer Las-4000 (Fuji-Film). Quantification of the scanned images was performed using the MultiGauge program (Fuji-Film) according to the manufacturer’s instructions. The StAR, 11β-hydroxylase or P450scc protein signal was corrected to the β-actin signal.

**RIA of corticosterone.** The concentrations of corticosterone in the media were determined by RIA as previously described (Chang et al., 2002; Lo et al., 1998). The RIA was established for the measurement of plasma corticosterone levels with this antiserum (PSW#4–9). The sensitivity of corticosterone RIA was 5 pg/tube, and the intra- and inter-assay coefficients of variation were 3.3% (n = 4) and 9.2% (n = 4), respectively.

**RIA of pregnenolone.** The concentration of pregnenolone in the media was determined by RIA as previously described (Kau et al., 1999b). The sensitivity of the pregnenolone RIA was 16 pg/tube, and the
intra- and inter-assay coefficients of variation were 2.3% (n = 6) and 3.7% (n = 4), respectively.

**Statistical analysis.** Results were expressed as mean ± SEM. Treat means were tested for homogeneity using an analysis of variance, and difference between specific means was performed by Student’s t-test, or ANOVA followed by Duncan’s test (Steel and Torrie, 1960). A value of $p < 0.05$ was interpreted as statistically significant.

**RESULTS**

Due to complexity of the study, the results are organized into two parts. The first part examined the effects of NP on steroidogenesis. The results are organized in Figures 1–5. The second part examined the mechanisms associated with NP effects. The results are summarized in Figures 6 and 7.

**Effects of NP on Corticosterone Release Induced by ACTH, 8-Br-cAMP, IBMX, or IBMX + FSK**

Incubation of ZFR cells with ACTH (10^{-10} M), 8-Br-cAMP (2 × 10^{-3} M), IBMX, or IBMX + FSK (2.5 × 10^{-3} M) for 1 h increased ($p < 0.01$) the corticosterone release (Figs. 1 and 2). Co-incubation of ACTH, 8-Br-cAMP, IBMX, or IBMX + FSK with NP (14.2–42.5 μM) resulted in a dose-related increase in ACTH-, 8-Br-cAMP-, IBMX-, or IBMX + FSK-induced corticosterone release by ZFR cells (Fig. 1 or Fig. 2, $p < 0.05$ or $p < 0.01$). At 85 μM, NP still stimulated the vehicle, ACTH-, IBMX-, or IBMX + FSK-induced corticosterone release by ZFR cells (Figs. 1 and 2, $p < 0.01$); however, it did not stimulate 8-Br-cAMP-induced corticosterone release.

**Effects of NP on the Biosynthesis Pathway of Corticosterone**

To investigate the effect of NP on the steroidogenesis of ZFR cells, the ZFR cells were incubated with NP (0 or 14.2–85 μM) combined with 10^{-5} M 25-OH-cholesterol, a substrate of P450scc, 10^{-7} M pregnenolone, a substrate of 3β-HSD, 10^{-6} M progesterone, a substrate of 21-hydroxylase, or 10^{-6} M deoxycorticosterone, a substrate of 11β-hydroxylase. The NP (85 μM) showed a dose-related stimulation of corticosterone release induced by 25-OH-cholesterol, pregnenolone, progesterone, and deoxycorticosterone (Fig. 3, $p < 0.01$).

To investigate the effects of NP on P450scc, 25-OH-cholesterol (10^{-5} M) with triostane (5 × 10^{-6} M) was used to challenge the ZFR cells (Fig. 4). The NP (85 μM) was observed to stimulate the vehicle, triostane, and triostane combined with 25-OH-cholesterol pregnenolone release (Fig. 4, $p < 0.01$).

For kinetic analysis of NP on 11β-hydroxylase, cells after preincubation were incubated for 1 h with or without NP (85 μM) in the presence of deoxycorticosterone (2 × 10^{-7} to 10^{-7} M, the substrate of 11β-hydroxylase). After incubation and centrifugation at 200 × g for 10 min, the supernatant was used to measure the concentration of corticosterone by RIA.

NP (85 μM) significantly stimulated deoxycorticosterone (2 × 10^{-7} to 10^{-6} M)-induced corticosterone production from ZFR cells in Figure 5. The $K_M$ and $V_{max}$ were derived from a double-reciprocal plot of corticosterone production rate versus deoxycorticosterone (2 × 10^{-7} to 10^{-5} M). An apparent difference was observed on the $K_M$ between the control group (7.404 μM) and the NP group (2.952 μM). For the $V_{max}$, no apparent difference between the control group (362.50 ng/5 × 10^4 cells/h) and the NP group (337.31 ng/5 × 10^4 cells/h) was observed.

**Effects of ICI182780 on ACTH ± NP-Induced Corticosterone Release**

To assess whether NP stimulated ACTH-induced corticosterone release through the ER, ZFR cells were treated with the specific ER antagonist, ICI182780 (5 μM), for 30 min prior to NP and ACTH stimulation. Our results revealed that treatment with NP (42.5 μM) increased ACTH-stimulated corticosterone release (Fig. 6, $p < 0.01$). ICI182780 was found to inhibit ACTH + NP-induced corticosterone release ($p < 0.01$), but not ACTH-stimulated corticosterone release. These results suggest that NP might act through ERs to stimulate corticosterone release from rat ZFR cells.

*FIG. 1. Effects of NP (0 or 14.2–85 μM) on the vehicle, ACTH- (10^{-10} M), and 8-Br-cAMP- (2 × 10^{-5} M) stimulated corticosterone release by ZFR cells in male rats. *$p < 0.05$, **$p < 0.01$ when compared with NP = 0M within the respective group. ++$p < 0.01$ when compared with the vehicle group. Each value represents mean ± SEM.
Effects of NP on ACTH ± SNP- or ACTH ± SB203580-Induced Corticosterone Release

To assess whether NP stimulated ACTH-induced corticosterone release through an NO mediator or the p38 MAPK pathway, ZFR cells were treated with SNP (0.1–0.5 mM, a specific NO generator) or SB203580 (10⁻⁶ to 10⁻⁵ M, an inhibitor of p38 MAPK) for 30 min prior to NP and ACTH stimulation. An NP concentration of 42.5 µM was found to stimulate ACTH- or ACTH + SNP-induced corticosterone release from ZFR cells (Fig. 7, upper panel, *p < 0.01). SNP was observed to inhibit ACTH-induced corticosterone release (Fig. 7, upper panel, *p < 0.01) but had no effect when ACTH was combined with NP. These results suggest that the NO generator could inhibit corticosterone release, and NP-stimulated corticosterone release from rat ZFR cells might be independent of an NO mediator. SB203580 could inhibit ACTH-induced corticosterone release from ZFR cells in dose dependent (Fig. 7, lower panel, *p < 0.01). The decreasing corticosterone quantity was 12.42 ng/50,000 cells/h when the cells were stimulated by ACTH + SB203580, but it was 10.51 ng/50,000 cells/h when the cells were stimulated by ACTH + SB203580 combined with NP (Fig. 7, lower panel, *p < 0.01). The decreasing corticosterone quantity was 12.42 ng/50,000 cells/h when the cells were stimulated by ACTH + SB203580, but it was 10.51 ng/50,000 cells/h when the cells were stimulated by ACTH + SB203580 combined with NP (Fig. 7, lower panel, *p < 0.01). These results suggest that ACTH mediated through p38 MAPK pathway to stimulate corticosterone release from ZFR cells. However, NP-stimulated corticosterone release from rat ZFR cells might be independent of p38 MAPK pathway.

Protein Expression of StAR, 11β-hydroxylase, and P450scc

Bands of StAR (at 30, 32, and 37 kDa), 11β-hydroxylase (at 51.5 kDa), and P450scc (at 54 kDa) were detected in the rat ZFR cells (Fig. 8). The β-actin signal (45 kDa) was used as an internal control. Three repeated experiments showed a similar pattern. ZFR cells were incubated with NP (0 or 42.5 µM) in the presence of FSK (2 x 10⁻⁵ M) or ACTH (10⁻⁸ M) for 1 h. NP cannot affect the StAR, 11β-hydroxylase, or P450scc protein expression in the FSK group or ACTH group (Fig. 8).

DISCUSSION

Since the book of Silent Spring (Carson, 1962), world has gradually realized a lot of man-made chemicals which
intending to advance human civilization are also environmental toxic, i.e., dichlorodiphenyltrichloroethane, diestylstilbestrol (DES), bisphenol A, phthalate etc. The study of these endocrine-disrupting chemicals is mainly focus on the reproductive function, i.e., xenoestrogenic effect (Chen et al., 2009; Blair et al., 2000; White et al., 1994). We have reported estradiol enhanced corticosterone secretion from ovariectomized rat (Lo et al., 2000). The presence of ER in adrenal cortex (de Cremoux et al., 2008) and estrogenic property of NPs suggested possible direct effect of NP on corticosterone secretion. The effect of NP on the corticosterone secretion becomes inquisitive with the realization of alkylphenolic products presence in human and infant food source, i.e., fish, eggs, vegetables, fruits, meats, rice, and human milk (Chen et al., 2010; Ademollo et al., 2008; Shao et al., 2007). Especially NP can cross the placenta and cause reproductive and developmental toxicity (Bechi et al., 2006; Hong et al., 2004). It reminds DES tragedy of public health (Swan, 2000). The extensive application of alkylphenols could turn to be a public health issue. Adrenal secretion is part of body response mechanism to stress. It is logical that the study of environmental toxic chemicals need extend to examine the impact of these chemicals upon adrenal function (Hinson and Raven, 2006).

The examination of the effect of NP on corticosterone steroidogenesis includes ACTH, 8-Br-cAMP, IBMX, and FSK, and the chain of substrates for corticosterone production is examined in Figures 1–5. By the examination of corticosterone production pathway, the stimulation process from ACTH, second messenger cascade system, to steroidogenetic enzyme substrates supplementation, were examined with/without NP presence. The additive/synergistic effect of NP with ACTH and second messenger cascade, i.e., FSK, 8-Br-cAMP, and IBMX, suggest the action point is beyond the stimulation level. By following the logical flow, examination of key enzyme activities was carrying on. The four examined key enzymes are P450scc, 3\(\beta\)-HSD, 21\(\beta\)-hydroxylase, and 11\(\beta\)-hydroxylase. NP significantly increased the pregnenolone in the absence or presence of 3\(\beta\)-HSD inhibitor (trilostane) suggested that P450scc might be one of the effecting points of NP. The significant increased corticosterone secretion by progesterone and deoxycorticosterone suggested 11\(\beta\)-hydroxylase is another effecting point of NP. The significant increased corticosterone secretion by progesterone and deoxycorticosterone suggested 11\(\beta\)-hydroxylase is another effecting point of NP. NP could decrease Michaelis constant but not maximum velocity (Fig. 5) and 11\(\beta\)-hydroxylase protein expression (Fig. 8), therefore NP might increase 11\(\beta\)-hydroxylase activity.

StAR protein, a mitochondrial protein, was purified, cloned, and sequenced by Clark et al. (1994). Its role has been
proposed for the transport of cholesterol to the inner mitochondrial membrane and to the site of cytochrome P450scc in steroidogenesis (Clark et al., 1994; Lin et al., 1995). The 30 kDa mitochondrial protein arises from two precursor proteins with molecular masses of 37 and 32 kDa (Stocco and Sodeman, 1991). After the import and processing of the precursor proteins in mitochondria, cholesterol is quickly converted to pregnenolone by P450scc. The cleavage of the precursor protein by the matrix processing protease result in the formation of mature 30 kDa form of the StAR protein (Stocco, 2000). These reports seem to suggest that StAR is rapidly synthesized in the cytosol in response to hormone stimulation.

In the present study, the protein expressions of StAR (at 30, 32, and 37 kDa) and P450scc were examined by Western blot (Fig. 8). The result of Western blot demonstrated that NP did not significantly affect StAR (neither mature nor precursor protein) or P450scc protein expression (Fig. 8). When the ZFR cells were incubated with FSK more than 2 h, FSK could increase the mature StAR protein expression (data not shown).

The results of mechanisms associated with NP effects are organized in Figures 6 and 7. NP has weak estrogenic activity (Schwaiger et al., 2002; Van den Belt et al., 2004). ER antagonist ICI182780 was used to verify whether the NP effect mediated through ER or not. In the presence of ACTH, ICI182780 significantly inhibited the NP effect but not ACTH-alone group. These results strongly indicate that effect of NP mediated through ERs to stimulate corticosterone release. It has been shown that NO inhibits steroidogenesis (Van Voorhis et al., 1994; Dave et al., 1997; Del Punta et al., 1996). Cymeryng et al. (1998, 1999, 2002) proposed that NO may be a negative modulator of adrenal zona fasciculata or cell line Y1 steroidogenesis. The results in the study agree with the concept of NO inhibit corticosterone secretion and also reveal the effect of NP is not mediated through NO. NP is an environmental xenoestrogen. The JNK and p38 MAPK pathways are believed to be more responsive to environmental and physiochemical stresses. So the possibility of NP effect mediated through p38 MAPK examined. The p38 MAPK inhibitor (SB203580) significantly interfere ACTH stimulation effect but has no effect on the NP stimulation effect on corticosterone secretion. Furthermore, NP could not affect FSK or ACTH-induced phosphorylated and total p38 proteins expression (data not shown). These results demonstrate the effect of NP is not mediated through p38 MAPK. By these considerations and examined results, the effect of NP is only mediated through ER, which cascade through P450scc and 11β-hydroxylase.

As far as we know, this is the first study that examines the effect of NP on corticosterone secretion. The evidence from this study shows NP significantly increases corticosterone secretion. These results provide a new consideration of environmental estrogens impact upon public health. With wide application of APEO, human may exposed to NP via food, water, contact, respiration ... etc. Chronic providing mice with corticosterone in drinking water resulted in rapid and dramatic weight gain, increased adiposity, and elevated plasma leptin and insulin levels (Karatosoreos et al., 2010). One possible health related issue could be non-physiological increase of serum corticosterone due to NP exposure. Chronic elevated...
non-regulated concentration of corticosterone could stir up metabolic syndrome, i.e., Cushing’s syndrome, obesity. Recently, environmental endocrine disrupting chemicals have been shown to influence adipogenesis and obesity (Grünn and Blumberg, 2009; Newbold et al., 2009). Obesogens can be defined functionally as chemical agents that inappropriately regulate and promote lipid accumulation and adipogenesis (Grünn and Blumberg, 2009). Chen et al. (2009) reviewed the disturbance of estrogenic obesogens on the regulation of energy metabolism pathways and concluded glucose transport, glycolysis, tricarboxylic acid cycle, mitochondria respiratory chain, and fatty acid β-oxidation, all metabolic pathways were potentially disturbed by obesogens. Those disturbance in energy metabolism pathways resulted in obesity. Karatosoreos et al. (2010) also indicates environmental estrogen, i.e., NP can increase the corticosterone release which can result in obesity. The prevalence of obesity is a world epidemic issue (CDC, 2008; Ogden et al., 2002). The widespread application of APEO and its main degradation product, NP, might connect to this public health issue. In addition to the estrogenic activity of obesogens disturbing energy metabolism pathways, we also suggest the obesogens induced corticosterone secretion, which deteriorates the impact metabolic syndrome and obesity. The other worrisome aspect is the NP detected in the human milk. It indicates the NP exposure could be at in utero and neonatal stage. Kaufman et al. (2007) reported that early life stress resulted in increasing visceral fat and incidence of metabolic syndrome later in life. By the “developmental origins of health and disease theory” (Gluckman and Hanson, 2004), maternal/infant NP exposure could have long subsequent consequences. It indicates the importance and urgency to study the impact environmental NP on human public health.

In summary, the results demonstrate that NP stimulates corticosterone secretion through ER and increase the enzyme activities of P450scc and 11β-hydroxylase. The estrogenic activity suggests the extensive application of APEO could be one of the responsible reasons for world epidemic obesity. The study of NP is not only just environmental toxicity issue. It is also an important public health issue.

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![FIG. 8. Effects of NP on the synthesis of the StAR, 11β-hydroxylase, and P450scc proteins by ZFR cells stimulated with FSK or ACTH. Western blot analysis of cell extracts subjected to SDS-PAGE and developed by enhanced chemiluminescence. StAR (30, 32, and 37 kDa), 11β-hydroxylase (51.5 kDa), or P450scc (54 kDa) was detected by incubation with StAR, 11β-hydroxylase, or P450scc antisera. β-actin served as an internal control.](image-url)


