MINIREVIEW

Endocrine Disruption by Cadmium, a Common Environmental Toxicant with Paradoxical Effects on Reproduction

MICHAEL C. HENSON*,[1] AND P. JORGE CHEDRESE†

*Departments of Obstetrics and Gynecology, Physiology, Structural and Cellular Biology, and the Interdisciplinary Program in Molecular and Cellular Biology, Tulane University Health Sciences Center, New Orleans, Louisiana 70112; and †Department of Obstetrics, Gynecology and Reproductive Sciences, University of Saskatchewan, Saskatoon, Saskatchewan S7N OW8, Canada

Cadmium (Cd^{2+}) is a common environmental pollutant and a major constituent of tobacco smoke. Exposure to this heavy metal, which has no known beneficial physiological role, has been linked to a wide range of detrimental effects on mammalian reproduction. Intriguingly, depending on the identity of the steroidogenic tissue involved and the dosage used, it has been reported to either enhance or inhibit the biosynthesis of progesterone, a hormone that is inexorably linked to both normal ovarian cyclicity and the maintenance of pregnancy. Thus, Cd^{2+} has been shown to exert significant effects on ovarian and reproductive tract morphology, with extremely low dosages reported to stimulate ovarian luteal progesterone biosynthesis and high dosages inhibiting it. In addition, Cd^{2+} exposure during human pregnancy has been linked to decreased birth weights and premature birth, with the enhanced levels of placental Cd^{2+} resulting from maternal exposure to industrial wastes or tobacco smoke being associated with decreased progesterone biosynthesis by the placental trophoblast. The stimulatory effects of Cd^{2+} on ovarian progesterone synthesis, as revealed by the results of studies using stable porcine granulosa cells, appear centered on the enhanced conversion of cholesterol to pregnenolone by the cytochrome P450 side chain cleavage (P450scc). However, in the placenta, the Cd^{2+}-induced decline in progesterone synthesis is commensurate with a decrease in P450scc. Additionally, placental low-density lipoprotein receptor (LDL-R) mRNA declines in response to Cd^{2+} exposure, suggesting an inhibition in the pathway that provides cholesterol precursor from the maternal peripheral circulation. Potential mechanisms by which Cd^{2+} may affect steroidogenesis include interference with the DNA binding zinc (Zn^{2+})-finger motif through the substitution of Cd^{2+} for Zn^{2+} or by taking on the role of an endocrine disrupting chemical (EDC) that could mimic or inhibit the actions of endogenous estrogens. Divergent, tissue-specific (ovary vs. placenta) effects of Cd^{2+} also cannot be ruled out. Therefore, in consideration of the data currently available and in light of the potentially serious consequences of environmental Cd^{2+} exposure to human reproduction, we propose that priority should be given to studies dedicated to further elucidating the mechanisms involved. Exp Biol Med 229:383–392, 2004

Key words: cadmium; placenta; stable granulosa cells; steroidogenesis; toxicology

Cadmium (Cd^{2+}) is a heavy metal that is dispersed throughout the modern environment mainly as a result of pollution from a variety of sources (1, 2). Much of that released into the environment in recent years can be traced to occupational exposure and the wastes associated with mining, smelting, and electroplating as well as the intensive use of consumer products such as nickel/Cd^{2+} batteries, pigments, and plastics (2, 3). Its high concentrations in the soil and water supply has made it easily detectable in meat, fish, and fruits, although tobacco smoke may be one of the most common sources of Cd^{2+} contamination in the general population (4). The metal has no known beneficial biological function and prolonged exposure to it has been linked to toxic effects in both humans and animals (3). Cd^{2+} has a long biological half-life of 15–30 years (5), mainly due to its low rate of excretion from the body, and accumulates over time in blood, kidney,

This work was supported by the Tulane Department of Obstetrics and Gynecology for studies in the laboratory of MCH and by the Canadian Network of Toxicology Centers, the Natural Sciences and Engineering Research Council of Canada, the Saskatchewan Agricultural Development Fund, a J. Murray Research Grant, and a Clinical Teaching and a Research Grant from the College of Medicine, University of Saskatchewan, for support of studies conducted in the laboratory of P.J.C.

[1] To whom correspondence should be addressed at the Department of Obstetrics and Gynecology (SL-11), Tulane University Health Sciences Center, 1430 Tulane Avenue, New Orleans, LA 70112-2699. E-mail: henson@tulane.edu

1535-3702/04/2292-0001$15.00

and liver (1, 5–7) as well as in the reproductive organs, including the placenta, testis, and ovaries (3, 7–10). Specifically, human exposure to the metal is associated with increased incidences of renal disease, hypertension, osteoporosis, and leukemia, as well as cancers of the lung, kidney, urinary bladder, pancreas, breast, and prostate (11). The practically ubiquitous nature of Cd^{2+} and its deleterious effects on human health makes it a serious problem of worldwide scope and has lead, therefore, to numerous patient-based investigations by biomedical researchers throughout Asia (11–13), the Middle East (14), Australia (15), Europe (3, 7–9, 16), and the Americas (17–19).

Cd^{2+} Exposure and Reproduction

Various effects of Cd^{2+} on reproductive endocrinology have been described, but definitive conclusions about its actions on target tissues vary depending on the experimental model and the dosage employed. Exposure of rodents to the metal resulted in a down-regulation of pituitary hormones; including gonadotropins, prolactin, ACTH, growth hormone, and thyroid-stimulating hormone (20). Similarly, in pseudopregnant rats and in cultured granulosa cells from both rats and humans, Cd^{2+} inhibited progesterone synthesis (10, 21, 22). Because tobacco smoke is a source of Cd^{2+} contamination, the reproductive organs of smokers are generally considered to be at increased risk of exposure to toxic levels of Cd^{2+}. Thus, enhanced Cd^{2+} concentrations and lower progesterone levels were evidenced in the follicular fluid and in the placentas of smokers compared with those of nonsmokers (4, 8), and exposure of hamsters to realistic environmental dosages of tobacco smoke had severe repercussions on reproductive structures. Exposure was linked to a decline in the number of corpora lutea, as well as a reduction in their vascular area. It caused blebbing of the ciliated cells in the oviductal epithelium and resulted in a decrease in the ratio of ciliated to secretory cells in the ampulla. Exposure also resulted in decreased uterine length and an increased number of uterine implantation sites (23). Although the results of studies conducted in cultured human trophoblast cells indicated that Cd^{2+} inhibited placental progesterone synthesis (24–26), some reports suggest that, in many instances, Cd^{2+} stimulates steroidogenesis. Thus, Cd^{2+} administrated to female rats during estrus and diestrus resulted in increased serum progesterone levels (10, 27) and stimulated progesterone synthesis in both cultured porcine granulosa cells (6) and JAr choriocarcinoma cells, a malignant trophoblast cell line (28). Therefore, although the results of many studies concur in suggesting a significant impact on reproduction, the specific mechanisms of action on steroidogenesis are still subject to conjecture.

Effects of Cd^{2+} in the Ovary

The stimulatory and inhibitory effects of Cd^{2+} on progesterone synthesis (see Fig. 1) were recently investigated using the steroidogenically stable JC-410 porcine granulosa cell line (29), which was genetically modified with gene constructs containing the promoter region of the cytochrome P450 side chain cleavage (P450scc) gene linked to a luciferase reporter gene (30). P450scc is a hormonally regulated rate-limiting steroidogenic enzyme that catalyzes the conversion of cholesterol into pregnenolone, the immediate precursor of progesterone (31). It was observed that low (0.6–3 μM) and high (5 μM) concentrations of $CdCl_2$ in the culture media had opposite effects on the P450scc promoter activity (32). Although these concentrations are somewhat arbitrary by nature, dosages were chosen from the lower ranges of previous *in vivo* and *in vitro* studies (as reviewed, 16). As illustrated in Figure 2, at low concentrations, $CdCl_2$ stimulated P450scc gene promoter activity in a dose- and time-dependent fashion; while at high concentrations, it inhibited P450scc gene promoter activity. Low concentrations of $CdCl_2$ increased P450scc mRNA levels and progesterone synthesis but did not affect cell count, protein content, or cellular morphology in cultured nontransfected JC-410 cells. High concentrations of $CdCl_2$ inhibited P450scc gene promoter activity and caused a reduction in cell number and cellular protein content as well as changes in cell morphology. Overall, results suggested that Cd^{2+} exerted a dual action in granulosa cells; low concentrations activated, while high concentrations inhibited expression of the P450scc gene. These studies were conducted in serum-free culture conditions in which $CdCl_2$ stimulated expression of the P450scc gene and progesterone synthesis. Therefore, because synthesis of progesterone in JC-410 stable granulosa cells is entirely dependent on an intracellular source of cholesterol (33), it is reasonable to speculate that the effects of Cd^{2+} were independent of changes in the metabolism of low-density lipoprotein (LDL) or LDL receptor (LDL-R) function. This may explain why Cd^{2+} also elevates progesterone levels in estrous and diestrous rats (10, 27) in cultured porcine granulosa cells (6) and in choriocarcinoma cells (28). Effects in these studies were observed at Cd^{2+} concentrations that were dramatically lower than the concentrations (5–20 μM) reported to induce endocrine disruption in cultured human trophoblasts (24–26). Such lower concentrations may represent typical tissue burdens to which humans and animals are commonly exposed. Based on these results, therefore, it is reasonable to conclude that exposure to even low concentrations of Cd^{2+} is sufficient to significantly affect the steroidogenic pathway.

Effects of Cd^{2+} During Pregnancy and in the Placenta

In human pregnancy, maternal exposure to Cd^{2+} is associated with low birth weight (34, 35) and an increased incidence of spontaneous abortion (36). Although pharmacokinetic studies have demonstrated that the metal does not readily reach the fetus, it accumulates in high concentrations

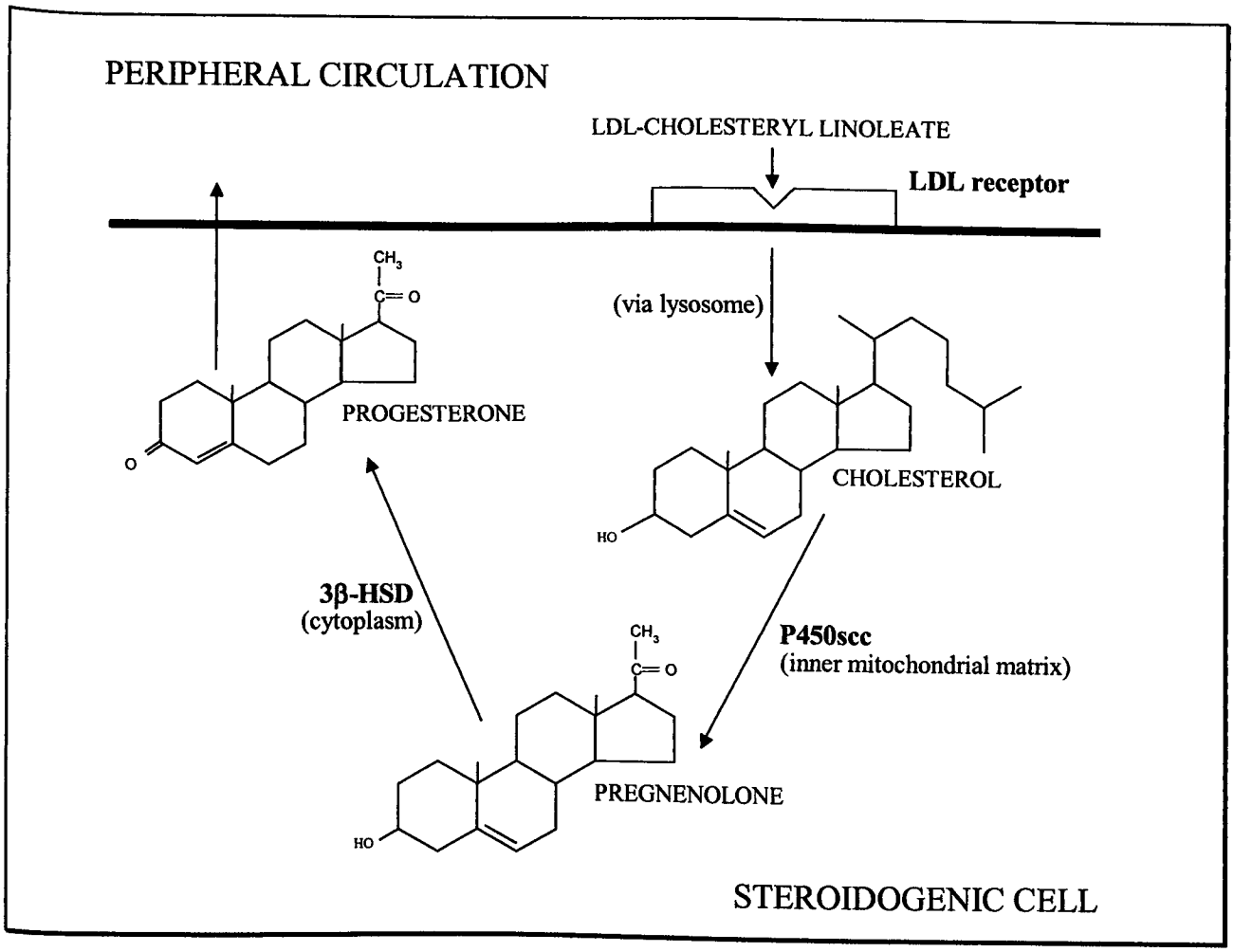

Figure 1. The progesterone biosynthetic pathway. Low density lipoprotein (LDL)-cholesteryl linoleate is bound to a specific LDL receptor and internalized. Lysosomal proteolysis yields free cholesterol. Cytochrome P450 side chain cleavage (P450scc), on the inner mitochondrial matrix, converts it to pregnenolone. In the cytoplasm, pregnenolone is converted to progesterone by 3β-hydroxysteroid dehydrogenase (3β-HSD). Cholesterol may also be produced *de novo* from acetate in many cell types, although in the placental trophoblast, this mechanism is down-regulated.

in the placenta (8, 37–39). In both perfused human placentae (40) and *in vivo* rodent studies (41), observable symptoms of Cd^{2+} toxicity included lysosomal vesiculation, nuclear chromatin clumping, and mitochondrial calcification in trophoblast cells. In perfused human placentae, exposure to Cd^{2+} also precipitated a decline in the secretion of human chorionic gonadotropin (hCG; Ref. 40), a polypeptide hormone vital to early pregnancy maintenance. The placental trophoblast, which is the site of hCG synthesis, is also responsible for the production of progesterone (42), a steroid hormone that plays a vital role in pregnancy maintenance by promoting uterine myometrial quiescence (43). The potential for environmental Cd^{2+} to affect placental progesterone production by Cd^{2+} was evidenced by Piasek *et al.* (8), who determined an almost twofold increase in Cd^{2+} that was commensurate with an almost 50% decline in progesterone in the placentas of women who smoked during pregnancy. It is interesting to note, therefore, that maternal exposure to high levels of environmental Cd^{2+} led to a significant increase in the incidence of premature delivery in the Jinzu River basin in Japan, where itai-itai disease, the most severe manifestation of chronic Cd^{2+} poisoning is still reported (13). Indeed, older women with higher body burdens of the metal are at a greater risk for placental Cd^{2+} accumulation and subsequent adverse pregnancy effects, as maternal reserves may accumulate in placenta during pregnancy (16). Therefore, in a study investigating Cd^{2+} levels in maternal, umbilical cord, and newborn's blood following environmental exposure, maternal and newborn blood Cd^{2+} levels were not correlated with one another, while cord levels (as an index of placental burden) were highly correlated with maternal Cd^{2+} concentrations, which were significantly elevated in smoking mothers (19). This phenomenon, which has been replicated in rodents that exhibited a 16% increase in placental Cd^{2+} burden following just 5 days of inhalation exposure to environmental tobacco smoke (44), may help to explain the increased incidence of premature delivery in smoking

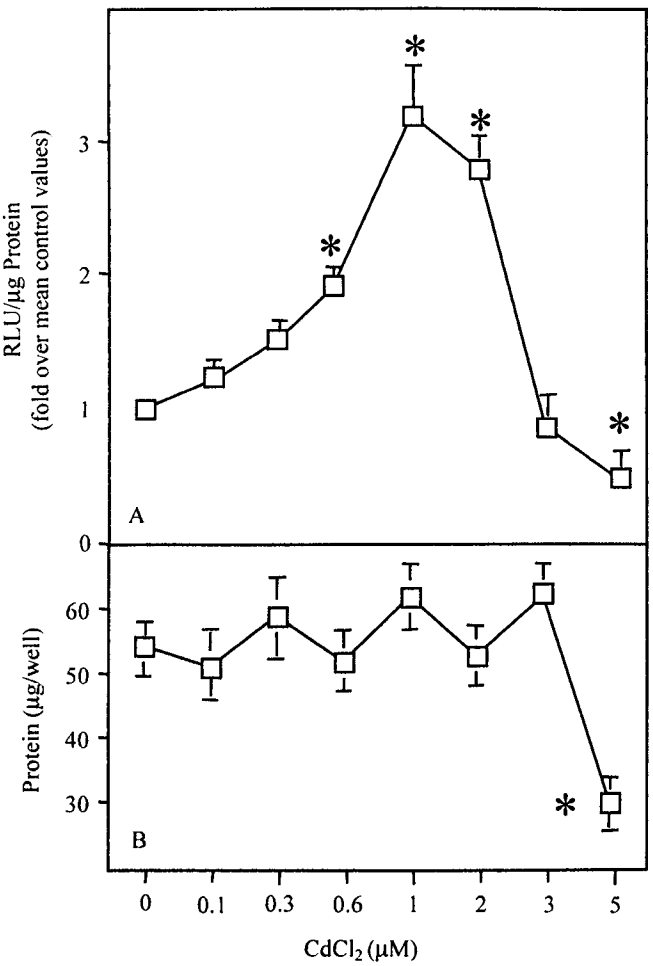

Figure 2. Effects of $CdCl_2$ on JC-410 cells stably transfected with P450scc-2320-LUC. Cells were exposed to the indicated concentrations of $CdCl_2$. After 24 hrs incubation, cells were collected and assayed for luciferase activity. (A) Data of relative light units (RLU) divided by (B) total protein content were expressed as a fold over mean control value for each treatment and analyzed by one-way ANOVA, followed by Fischer least significant difference test. Each point represents the mean ± SEM of three independent replications. Asterisk represents $P < 0.05$. Originally published in Smida *et al.*, Biology of Reproduction 70:25–31, 2004.

women. Indeed, high concentrations of placental Cd^{2+} that are directly related to maternal smoking have also been associated with an increase in trophoblast thickness, along with other quantifiable changes in placental morphology (17). In addition, Egawa *et al.* (45) determined that rats exposed to cigarette smoke between Days 14 and 16 of pregnancy exhibited an enhanced frequency of oxytocin-induced uterine myometrial contractions compared with rats breathing room air. The authors concluded that the effects of smoking on myometrial contractile activity could contribute to the increased risk of premature delivery reported in smoking mothers. With regard to this and related mechanisms, clinical studies have reported that both active (46) and passive exposure (47) to tobacco smoke during pregnancy constituted significant risk factors for preterm delivery, an effect that may be exacerbated among African Americans (48). In addition, tobacco smoke's effects on the placenta via similar Cd^{2+}-related mechanisms (36) might also be linked (49–51) to low birth weights and an enhanced incidence of intrauterine growth restriction (IUGR).

Collectively, therefore, clinical and experimental observations suggest that elevated concentrations of Cd^{2+} are at least one cause of the reduced levels of progesterone measured in the placentae of smokers, an effect that might contribute to premature delivery. Thus, it is possible that limited availability of cholesterol and/or inhibition of the steroidogenic genes could be mechanisms by which high Cd^{2+} concentrations contribute to inhibited progesterone synthesis, as described in the pseudopregnant rat model (21) and in cultured human (10) and rat (22) granulosa cells. To this end, we demonstrated that Cd^{2+} inhibited progesterone secretion in cultured human placental cells (5, 24) via a deleterious effect on LDL-R mRNA (25). However, because a number of steroidogenic enzymes are required for progesterone synthesis (see Fig. 1), it was quite possible that inhibition by Cd^{2+} might be multifaceted, affecting multiple sites in the steroidogenic pathway. Among the steroidogenic enzymes expressed by the primate placenta and involved in progesterone synthesis, P450scc and 3β-hydroxysteroid dehydrogenase (3β-HSD) are vital components. P450scc catalyzes the conversion of cholesterol into pregnenolone, which is then converted into progesterone via 3β-HSD (52). Syncytiotrophoblasts, formed in culture (as *in vivo*) from cytotrophoblast progenitors, adapt to facilitate this transition (53–55). Therefore, we continued our studies by investigating the influence of Cd^{2+} on the expression of the P450scc and 3β-HSD genes, and their enzymatic activities, in cultured human placental cells.

Progesterone secretion was reduced by coculture with $CdCl_2$, both prior to and during differentiation of cytotrophoblasts into syncytiotrophoblasts, or following completion of the differentiation process (5, 24–26). Cytotrophoblasts progressed to syncytiotrophoblastic maturity regardless of treatment, while treatment with $CdCl_2$ following differentiation inhibited 25-hydroxycholesterol (25-OHC)-stimulated progesterone secretion (26). Because 25-OHC readily traverses the plasma and mitochondrial membranes and taking into consideration that 3β-HSD is not a rate-limiting step in the steroidogenic pathway, the conversion of 25-OHC to progesterone represents an index of P450scc activity. Therefore, results indicated that Cd^{2+} did not interfere with progesterone synthesis/secretion by simply inhibiting morphological change but rather by exerting a direct inhibitory effect on the biosynthetic pathway. Moreover, no significant differences were observed in cell protein or lactate dehydrogenase (LDH) activity, an enzyme that serves as an indicator of cell injury and death, as a result of coculture with $CdCl_2$. These findings were reminiscent of our previous report of no significant Cd^{2+} induced decline in cell viability, as assessed by both DNA fragmentation assay and the conversion of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bro-

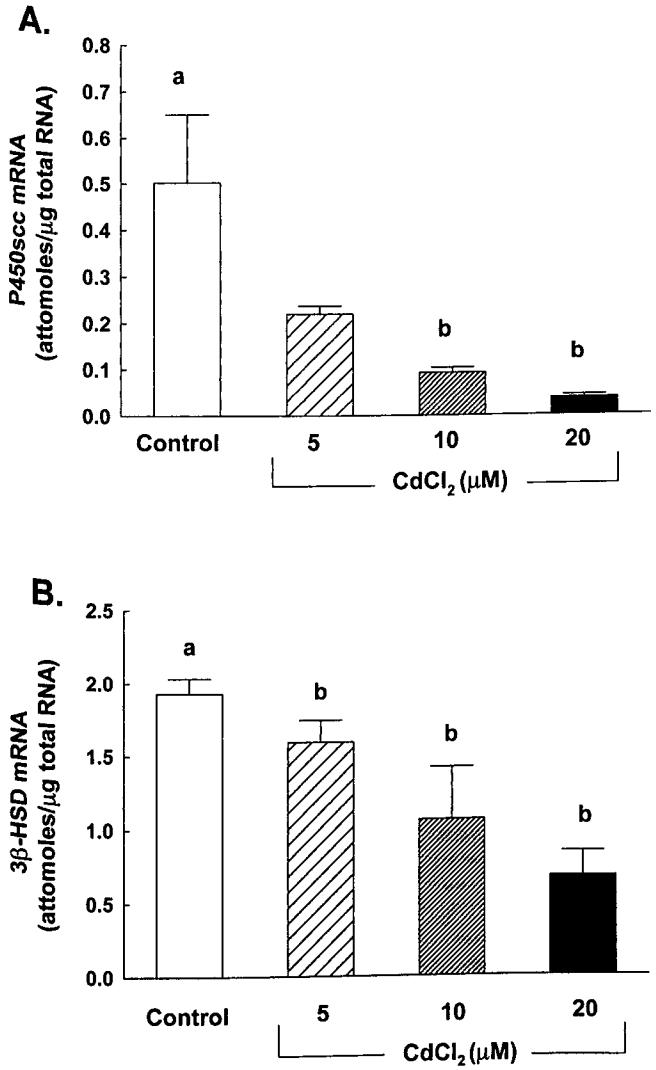

Figure 3. Effects of $CdCl_2$ on (A) P450scc and (B) 3β-HSD mRNA transcript abundance, as determined by competitive RT-PCR, in human trophoblasts cultured for 96 hrs. Values represent means ± SEM of four separate placental cultures. Different lowercase letters (a, b) indicate significant ($P < 0.05$) differences. Originally published in Kawai *et al.*, Biology of Reproduction 67:178–183, 2002.

mide (MTT) to formazan (25). Collectively, therefore, results suggested that Cd^{2+} suppression of progesterone synthesis in placental cell cultures could not be attributed to cell death by either apoptosis or necrosis. As illustrated in Figure 3, exposure to $CdCl_2$ caused a dose-dependent decline in P450scc and 3β-HSD mRNAs (26). Similarly, P450scc activity, as determined by the formation of progesterone from 25-OHC, was inhibited in cells cocultured with $CdCl_2$, indicating that P450scc is one site at which Cd might interfere with placental progesterone synthesis (Fig. 4). In this respect, it must be recognized that the formation of progesterone from 25-OHC did not reflect P450scc activity entirely, as its biosynthesis also included the conversion of pregnenolone to progesterone by 3β-HSD. However, in later experiments, basal progesterone synthesis was inhibited by the addition of aminoglutethimide, a potent inhibitor of P450scc (Fig. 5). Following the addition of 5 μg/ml pregnenolone over a short period, an approximately 500-fold increase in progesterone synthesis (i.e., 3β-HSD activity) was observed in the presence of 90 μM aminoglutethimide. However, coculture with 20 μM $CdCl_2$ had no significant effect on pregnenolone-stimulated progesterone synthesis, strongly suggesting that $CdCl_2$ did not inhibit 3β-HSD activity in cultured human trophoblasts.

Mechanism of Action of Cd^{2+}

The mechanisms by which Cd^{2+} affects cell function and gene expression were recently reviewed by Bhttacharyya *et al.* (1). Cd^{2+} can easily enter into the cells through the L-type voltage Ca^{2+} channels (56) and receptor-mediated Ca^{2+} channels (57) because both cations have similar radii size and charge (Ca^{2+} = 0.97 Å, Cd^{2+} = 0.99 Å). Cd^{2+} can also displace Ca^{2+} from its normal binding to calmodulin and protein kinase-C (PKC; Ref. 58). Calmodulin activates several enzymes of the second messenger pathways that regulate gene expression, including Ca^{2+}/calmodulin-dependent kinase, phosphodiesterase, and the myosin light-chain kinase (58). PKC is a family of Ser/Thr kinases that depends on Ca^{2+} and phospholipids to be activated (59). Cd^{2+} can activate PKC directly with a constant 5000-fold smaller than that of Ca^{2+} (60). Cd^{2+} concentrations between 0.1 and 5 μM interfere with the Ca^{2+}-ATPase pumps, leading to an immediate, transient, but substantial increase in intracellular Ca^{2+} (61, 62). This increase in Ca^{2+} results in the formation of inositol triphosphate (IP), which triggers the PKC signal cascade (63). It is known that an increase in the uptake of extracellular Ca^{2+} potentiates the effects of FSH and 8-Br-cAMP on transcription of the P450scc gene and progesterone synthesis in primary cultures of porcine granulosa cells (64). Therefore, it is reasonable to speculate that the low concentrations of Cd^{2+} may be sufficient to mimic the effects of Ca^{2+}, resulting in stimulation of the steroidogenic pathway and synthesis of progesterone.

The results of our studies in stable granulosa cells suggested that the effect of Cd^{2+} was specific in stimulating the promoter of the P450scc gene (Fig. 2) because $CdCl_2$ did not affect luciferase activity in cells transiently transfected with the promoterless version of the plasmid vector used in these experiments and had no effect on expression of the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Ref. 32). Moreover, $CdCl_2$ also stimulated activity of a construct containing only the first 100 base pairs of the promoter; suggesting that a *cis*-acting element located in the proximity of the transcription start site may be involved in the $CdCl_2$-stimulated expression of the porcine P450scc gene (30). They include consensus sequence motifs for the proto-oncogenes *c-jun* and *c-fos*, the cAMP regulatory element binding protein (CREB), the activator protein-1 (AP-1), and the specific protein-1 (Sp1). Exposure to Cd^{2+} activates these transcription factors in a variety of experimental models. In rat L6-myoblasts, 1 μM

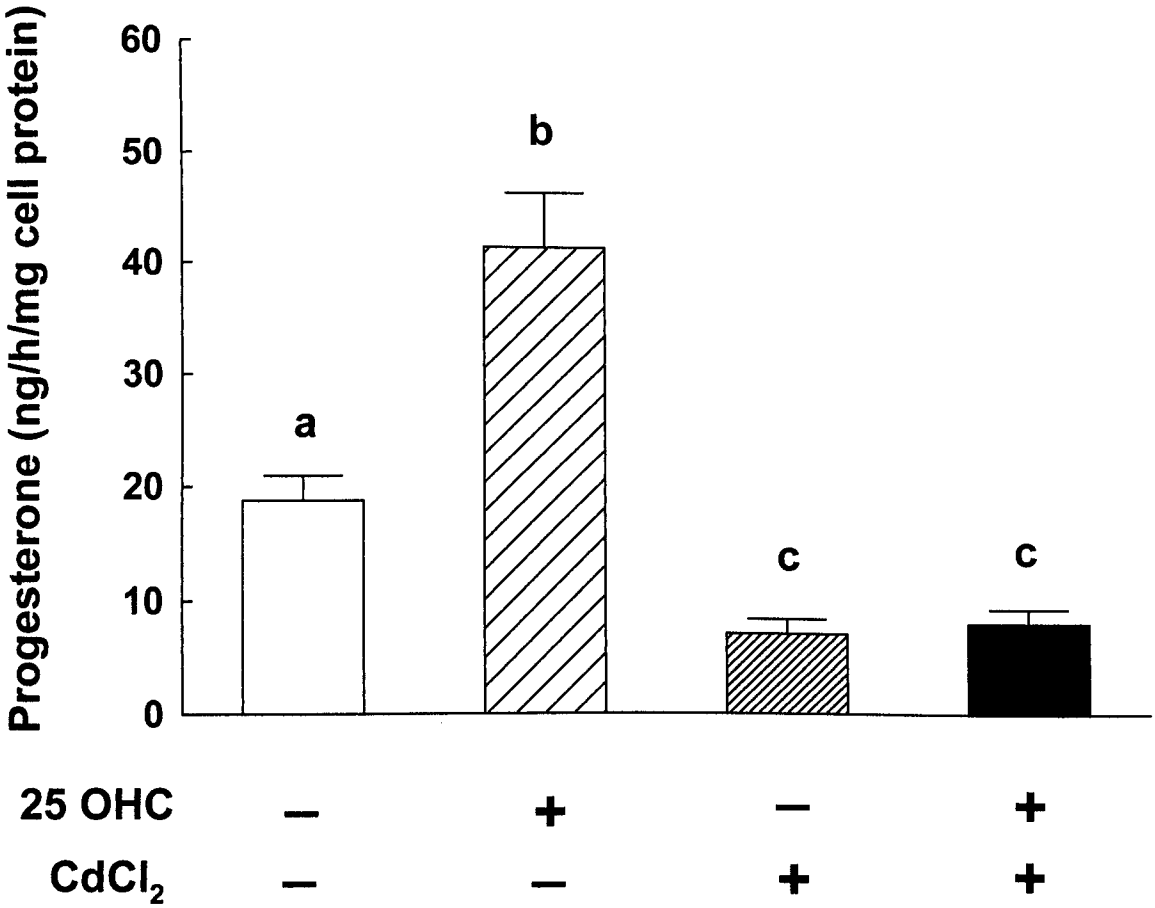

Figure 4. Effects of $CdCl_2$ on P450scc activity, as determined by the formation of progesterone from 25-hydroxycholesterol (25-OHC), in cultured human trophoblasts. Trophoblasts were incubated for 4 hrs (96–100 hrs in culture) with DMEM + 10% steroid-stripped FBS in the absence or presence of 20 μM $CdCl_2$ and/or 20 μg/ml 25-OHC. Values represent the means ± SEM of four separate placental cultures. Different lowercase letters (a, b, c) indicate significant ($P < 0.05$) differences. Originally published in Kawai *et al.*, Biology of Reproduction 67:178–183, 2002.

Cd^{2+} induces transcription of *c-jun* and *c-fos* through mechanisms mediated by PKC (65). It is known that, on stimulation, *c-jun* and *c-fos* form heterodimers that bind to AP-1 sites in several PKC-activated genes (66). Therefore, it is reasonable to speculate that PKC stimulation by Cd^{2+} may lead to activation of the P450scc genes through the AP-1 sites located in its promoter. Sp1 is a zinc-finger transcription factor originally defined in the promoter of the simian virus SV40 (67). Sp1 plays an essential role in eukaryotic gene expression, maintenance of homeostasis, cell cycle control, terminal differentiation, and apoptosis. The Sp1 binding motif was also found in other gene promoters, including those that are highly regulated by Cd^{2+}, such as human metallothionein IIA (68). Further investigation needs to be conducted to determine the mechanisms by which transcription factors mediate the effects of Cd^{2+} on the P450scc gene.

While it must be acknowledged that post-transcriptional regulation might also be at work, the apparent dose-dependent reduction in the abundance of P450scc mRNA transcripts in response to Cd^{2+} suggested an effect on transcriptional regulation. In this capacity, the hormonal regulation and developmental pattern of P450scc expression are specific to individual steroidogenic tissues. Thus, hormone receptor binding activates a G protein that increases intracellular cAMP, which in turn activates transcription of the P450scc gene (69, 70). Ringler *et al.* (71) reported that cAMP regulates progesterone synthesis in normal human trophoblasts, at least in part, by regulating the abundance of P450scc mRNA. Therefore, it is believed that human placental P450scc is regulated mainly at the level of gene expression and so it is possible that, in the trophoblast, Cd^{2+} inhibits progesterone synthesis by a direct interference with transcription.

Although a decline in 3β-HSD mRNA levels was also observed following coculture with $CdCl_2$, 3β-HSD activity was not significantly affected (26). Therefore, perhaps because of the typically high concentration of 3β-HSD within human trophoblasts (72), coculture with $CdCl_2$ exerted no significant effects on activity. It is also possible that 3β-HSD protein could be regulated at both transcriptional and post-transcriptional levels. In this capacity,

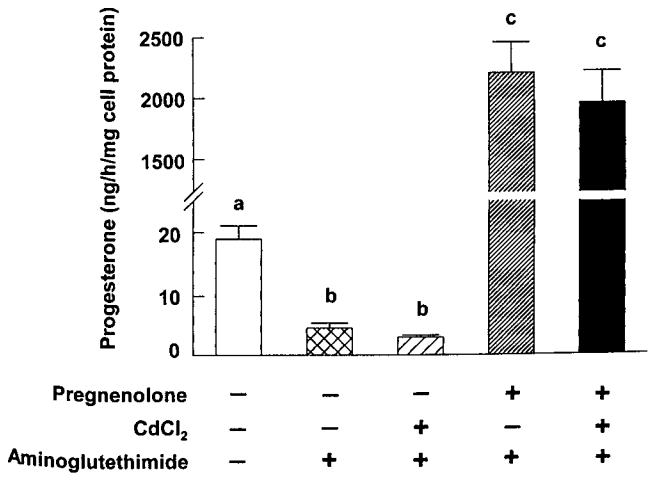

Figure 5. Effects of $CdCl_2$ on 3β-HSD activity, as determined by the formation of progesterone from pregnenolone in cultured human trophoblasts. Trophoblasts were incubated for 4 hrs (96–100 hrs in culture) with DMEM + 10% steroid-stripped FBS and 90 μM aminoglutethimide in the absence or presence of $CdCl_2$ and/or 5 μg/ml pregnenolone. Values represent the means ± SEM of five separate placental cultures. Different lowercase letters (a, b, c) indicate significant ($P < 0.05$) differences. Originally published in Kawai *et al*, Biology of Reproduction 67:178–183, 2002.

treatment of human trophoblasts with progesterone and estradiol increased 3β-HSD mRNA levels, but had no significant effect on protein levels (73), suggesting that 3β-HSD steady-state mRNA levels in human placenta could be regulated after transcription. A similar post-transcriptional regulatory mechanism has been suggested in respect to follicle-stimulating hormone-induced gene expression in rat granulosa cells (74). Therefore, Cd^{2+} may exert a direct effect on 3β-HSD transcript abundance but not on a post-transcriptional regulatory mechanism.

It has been proposed that accumulation of P450scc mRNA is mainly controlled by the cAMP-dependent pathway in human trophoblasts (72, 75). Thus, the human P450scc gene promoter contains consensus sequences that match known positive cAMP-responsive elements (76, 77). However, in our study (26), the cAMP analog, 8-bromo-cAMP, was not effective in blunting the decline in progesterone secretion elicited by Cd^{2+}. In addition, coculture with $CdCl_2$ did not influence the cAMP content in cultured cells, suggesting that cAMP in human trophoblasts may not be involved with the Cd^{2+}-induced inhibition of progesterone secretion. The possibility still exists, however, that Cd^{2+} interferes with the downstream cascade of the cAMP-protein kinase A-dependent pathway. Kostrzewska and Sobieszek (78) reported that higher Cd^{2+} concentrations inhibited the phosphorylation of myosin light chain kinase in the smooth muscle myosin, suggesting that Cd^{2+} interferes with the phosphorylation of protein kinases. An additional possibility is that the metal may directly affect transcription of the P450scc gene by interfering with the DNA binding zinc-finger motif through the substitution of Cd^{2+} for Zn^{2+} (79). Moreover, cations such as Cd^{2+} have been reported to alter the structure of nucleic acids (DNA, tRNA) and certain enzymes by reacting with their sulfydryl groups (80, 81). Therefore, it is possible that Cd^{2+} interferes with progesterone biosynthesis by one or more of the described mechanisms affecting transcription of the P450scc gene.

Estrogenic Effects of Cd^{2+}

Endocrine-disrupting chemicals (EDCs) are natural or synthetic agents that can mimic, enhance, or inhibit the action of endogenous hormones (82). The most commonly reported EDCs that affect reproduction are the pesticides that reproduce the effects of estrogens (83), and recent reports have also highlighted the potential of Cd^{2+} to mimic the effects of estrogen in various tissues. The first of these observations was made by Garcia-Morales *et al.* (84), in MCF-7 human breast cancer cells, who found that 1 pm Cd^{2+} mimicked the effect of estrogens by decreasing the level of estrogen receptor (ER) mRNA and transcription of the ER gene and increasing transcription of the progesterone receptor (PR) gene. The changes in steady-state levels of protein and mRNA of these genes were due to changes in transcription that were blocked by the antiestrogen, ICI-164384, thereby supporting the concept of an ER-mediated effect of the metal. Moreover, these estrogenic actions were not mimicked by Zn^{2+}, suggesting the existence of Cd^{2+}-specific effects on transcription (84). More recently, it was demonstrated that Cd^{2+} activates the ER-α through an interaction with the hormone-binding domain of the receptor, in which it binds with high affinity, blocking the binding of estradiol (85). The interaction of Cd^{2+} with the receptor appeared to involve several amino acids in the hormone-binding pocket of the receptor, suggesting that the metal may form a coordination complex with the hormone-binding domain and thereby activate the receptor (85). Similarly, *in vivo* studies conducted in rats showed that Cd^{2+} precipitated early puberty onset, increased uterine weight, and enhanced mammary development. Moreover, Cd^{2+} induced hormone-regulated genes in ovariectomized animals, including the PR and complement component C3 (86). In the mammary gland, Cd^{2+} promoted an increase in the formation of side branches and alveolar buds and the induction of casein, whey acidic protein, PR, and C3. Female offspring, exposed *in utero* to Cd^{2+}, experienced an earlier onset of puberty and an increase in the epithelial area and the number of terminal end buds in the mammary glands. Therefore, it might be concluded that Cd^{2+} mimics the effects of estrogens and that its effects cannot be extended to other heavy metals. To this end, the effects of Cd^{2+} appear to be mediated by the ERα and are independent of estrogen binding (87). Overall, the information presented provides strong evidence that Cd^{2+} is a potent nonsteroidal estrogen *in vivo* and *in vitro*.

Conclusions

We propose that, at low concentrations, Cd^{2+} stimulates transcription of the P450scc gene and the steroidogenic pathway in the ovary. We also propose that, at high concentrations, Cd^{2+} inhibits activity of the P450scc gene and progesterone synthesis in the ovary and facilitates changes in cell morphology and cell death. The effect of Cd^{2+} appears to be mediated via a *cis*-acting element located 100 bp upstream of the P450scc gene transcription start site. Conversion of cholesterol into pregnenolone by P450scc is an obligated step in the steroidogenic pathway. Therefore, the changes induced by Cd^{2+} on the expression of the P450scc gene in granulosa cells could affect the synthesis of all steroid hormones in the ovary. We also propose that cholesterol sequestration via the LDL-R and the conversion of cholesterol into pregnenolone by P450scc are two sites at which Cd^{2+} can interfere with progesterone production in cultured human trophoblasts. Further study is called for to definitively determine the collective mechanisms by which Cd^{2+} interferes with placental progesterone biosynthesis *in vivo* and to better understand their ramifications with respect to smoking, environmental exposure, normal placental function, and pregnancy maintenance.

Intriguingly, Cd^{2+} has been reported to affect steroidogenesis directly, both *in vivo* and *in vitro*, although differences in experimental methods and the concentrations tested may be responsible for variations in results (10, 21, 88, 89). With this in mind, the results of recent studies support the concept that, depending on its concentration, Cd^{2+} exerts dual effects on steroidogenesis. At low doses, Cd^{2+} stimulates DNA synthesis, cell multiplication, and malignant transformation (90, 91). Cd^{2+} administered in the mM range is toxic and can be associated with diminished DNA synthesis, apoptosis, and chromosome aberrations (92). When used in concentrations over 2–5 μM, Cd^{2+} induces apoptosis, partially via activation of the caspase-9 in HL-60 cells (93). Therefore, it is possible that the reduction in cell number, the cellular protein content, and the changes in cell morphology observed in our experiments with ovarian cells (32) were due to the apoptotic effects of high concentrations of $CdCl_2$. However, this does not appear to be the case in placenta, as trophoblast cells may possess an enhanced resistance to Cd^{2+}-induced cell death (9, 24–26).

To date, studies have been directed toward experiments to explain the paradoxical, dosage-dependent effects of Cd^{2+} on steroidogenesis; in the view that low doses upregulate, while comparatively high doses downregulate progesterone synthesis. Although when we consider that women with lower placental Cd^{2+} burdens also have lower placental progesterone levels and are prone to premature delivery, a negative effect of low Cd^{2+} concentrations on placental progesterone synthesis is suggested. In this capacity, therefore, it might be that the higher Cd^{2+} concentrations used in placental cell cultures for up to 96 hrs simply compensate for the lower placental Cd^{2+} burdens typically observed *in vivo*, to which the trophoblast is exposed for many months. Results might then be said to suggest a tissue-specific effect of Cd^{2+}, ovary versus placenta, on steroidogenesis. If this were the case, the most important question would become: Is the effect of Cd^{2+} on steroidogenesis really paradoxical at all?

We thank April G. O'Quinn, M.D., Gabriella Pridjian, M.D., Kenneth F. Swan, M.S., and Elease Bradford, M.S.P.H., of the Tulane University Health Sciences Center, Department of Obstetrics and Gynecology, for their support of studies conducted in the laboratory of MCH. The authors also gratefully acknowledge Mrs. Nathlynn Dellande and Mrs. Christine Meaden for their assistance in manuscript preparation.

1. Bhattacharyya MH, Wilson AK, Rajan SS, Jonah M. Biochemical pathways in cadmium toxicity. In: Zalup RK, Koropatnick J, Eds. Molecular Biology and Toxicology of Metals. London: Taylor and Francis, pp1–74, 2000.
2. Jarup L, Berglund M, Elinder CG, Nordberg G, Vahter M. Health effects of cadmium exposure—a review of the literature and risk estimate. Scand J Work Environ Health 24(Suppl 1):1–52, 1998.
3. Zadorozhnaja TD, Little RE, Miller RK, Mendel NA, Taylor RJ, Presley BJ, Gladen BC. Concentrations of arsenic, cadmium, copper, lead, mercury, and zinc in human placentas from two cities in Ukraine. J Toxicol Environ Health 61:255–263, 2000.
4. Zenzes MT, Krishnan S, Krishnan B, Zhang H, Casper RF. Cadmium accumulation in follicular fluid of women in *in vitro* fertilization-embryo transfer is higher in smokers. Fertil Steril 64:599–603, 1995.
5. Henson MC, Anderson MB. The effects of cadmium on placental endocrine function. Recent Res Dev Endocrinol 1:37–47, 2000.
6. Massanyi P, Uhrin V, Sirotkin AV, Paksy K, Forgács ZS, Toman R, Kovačik J. Effects of cadmium on ultrastructure and steroidogenesis in cultured porcine ovarian granulosa cells. Acta Vet Brno 69:101–106, 2000.
7. Varga B, Zsolnai B, Paksy K, Náray M, Ungváry GY. Age dependent accumulation of cadmium in the human ovary. Reprod Toxicol 7:225–228, 1993.
8. Piasek M, Blanusa M, Kostial K, Laskey JW. Placental cadmium and progesterone concentrations in cigarette smokers. Reprod Toxicol 15:673–681, 2001.
9. Fiala J, Hrubá D, Crha I, Rézl P, Totušek J. Is environmental cadmium a serious hazard to Czech population? Int J Occup Med Environ Health 14:185–188, 2001.
10. Paksy K, Rajczy K, Forgács Z, Lázár P, Bernard A, Gáti I, Kaáli GS. Effect of cadmium on morphology and steroidogenesis of cultured human ovarian granulosa cells. J Appl Toxicol 17:321–327, 1997.
11. Satoh M, Koyama H, Kaji T, Kito H, Tohyama C. Perspectives on cadmium research. Tohoku J Exp Med 196:23–32, 2002.
12. Moon C-S, Paik J-M, Choi C-S, Kim D-H, Ikeda M. Lead and cadmium levels in daily foods, blood and urine in children and their mothers in Korea. Int Arch Occup Environ Health 76:282–288, 2003.
13. Nishijo M, Nakagawa H, Honda R, Tanebe K, Saito S, Teranishi H, Tawara K. Effects of maternal exposure to cadmium on pregnancy outcome and breast milk. Occup Environ Med 59:394–397, 2002.
14. Sisman AR, Bulbul M, Coker C, Onvural B. Cadmium exposure in tobacco workers: possible renal effects. J Trace Elem Med Biol 17:51–55, 2003.
15. Satarug S, Baker JR, Reilly PE, Moore MR, Williams DJ. Changes in zinc and copper homeostasis in human livers and kidneys associated with exposure to environmental cadmium. Hum Exp Toxicol 20:205–213, 2001.

16. Piasek M, Kostial K, Laskey JW. Experimental studies on reproductive and perinatal effects of lead and cadmium. Environ Mgmt Health 7:29–32, 1996.
17. Bush PG, Mayhew TM, Abramovich DR, Aggett PJ, Burke MD, Page KR. A quantitative study on the effects of maternal smoking on placental morphology and cadmium concentration. Placenta 21:247–256, 2000.
18. Mielke HW, Gonzales CR, Smith MK, Mielke PW. The urban environment and children's health: soils as an integrator of lead, zinc, and cadmium in New Orleans, Louisiana, USA. Environ Res Sec A 81:117–129, 1999.
19. Galicia-Garcia V, Rojas-Lopez M, Rojas R, Olaiz G, Rios C. Cadmium levels in maternal, cord and newborn blood in Mexico City. Toxicol Lett 91:57–61, 1997.
20. Lafuente A, Cano P, Esquifino AI. Are cadmium effects on plasma gonadotropins, prolactin, ACTH, GH and TSH levels, dose-dependent? Biometals 16:243–250, 2003.
21. Piasek M, Laskey JW. Acute cadmium exposure and ovarian steroidogenesis in cycling and pregnant rats. Reprod Toxicol 8:495–507, 1994.
22. Piasek M, Laskey JW. Effects of *in vitro* exposure on ovarian steroidogenesis in rats. J Appl Toxicol 19:211–217, 1999.
23. Magers T, Talbot P, DiCarlantonio G, Knoll M, Demers D, Tsai I, Hoodbhoy T. Cigarette smoke inhalation affects the reproductive system of female hamsters. Reprod Toxicol 9:513–525, 1995.
24. Jolibois LS Jr., Shi W, George WJ, Henson MC, Anderson MB. Cadmium accumulation and effects on progesterone release by cultured human trophoblast cells. Reprod Toxicol 13:215–221, 1999.
25. Jolibois LS Jr., Burow ME, Swan KF, George WJ, Anderson MB, Henson MC. Effects of cadmium on cell viability, trophoblastic development, and expression of low density lipoprotein receptor transcripts in cultured human placental cells. Reprod Toxicol 13:473–480, 1999.
26. Kawai M, Swan KF, Green AE, Edwards DE, Anderson MB, Henson MC. Placental endocrine disruption induced by cadmium: effects on P450 cholesterol side-chain cleavage and 3β-hydroxysteroid dehydrogenase enzymes in cultured human trophoblasts. Biol Reprod 67:178–183, 2002.
27. Paksy K, Varga B, Lázár P. Zinc protection against cadmium-induced infertility in female rats. Effect of zinc and cadmium on the progesterone production of cultured granulosa cells. Biometals 10:27–35, 1996.
28. Powlin SS, Keng PC, Miller RK. Toxicity of cadmium in human trophoblast cells (JAr choriocarcinoma): role of calmodulin and the calmodulin inhibitor, zaldaride maleate. Toxicol Appl Pharmacol 144:225–234, 1997.
29. Chedrese PJ, Rodway MR, Swan CL, Gillio-Meina C. Establishment of a stable steroidogenic porcine granulosa cell line. J Molec Endocrinol 20:287–292, 1998.
30. Urban RJ, Shupnik MA, Bodenburg YH. Insulin-like growth factor-I increases expression of the porcine P-450 cholesterol side chain cleavage gene through a GC-rich domain. J Biol Chem 269:25761–25769, 1994.
31. Oonk RB, Krasnow JS, Beattie WG, Richards JS. Cyclic AMP-dependent and -independent regulation of cholesterol side chain cleavage cytochrome P-450 ($P\text{-}450_{scc}$) in rat ovarian granulosa cells and corpora lutea. cDNA and deduced amino acid sequence of rat $P\text{-}450_{scc}$. J Biol Chem 264:21934–21942, 1989.
32. Smida AD, Valderrama XP, Agostini MC, Furlan MA, Chedrese PJ. Cadmium stimulates transcription of the cytochrome P450 side chain cleavage gene in genetically modified stable porcine granulosa cells. Biol Reprod 70:25–31, 2004.
33. Rodway MR, Swan CL, Crellin NK, Gillio-Meina C, Chedrese PJ. Steroid regulation of progesterone synthesis in a stable porcine granulosa cell line: a role for progestins. J Steroid Biochem Molec Biol 68:173–180, 1999.
34. Kuhnert BR, Kuhnert PM, Zarlingo TJ. Associations between placental cadmium and zinc and age and parity in pregnancy women who smoke. Obstet Gynecol 71:67–70, 1988.
35. Frery N, Nessmann C, Girard F, Lafond J, Moreau T, Blot P, Lellouch J, Huel G. Environmental exposure to cadmium and human birthweight. Toxicology 79:109–118, 1993.
36. Shiverick KT, Salafia C. Cigarette smoking and pregnancy I: ovarian, uterine and placental effects. Placenta 20:265–272, 1999.
37. Wier PJ, Miller RK. The pharmacokinetics of cadmium in the dually perfused human placenta. Trophoblast Res 2:357–366, 1987.
38. Boadi WY, Yannai S, Urbach J, Brandes JM, Summer KH. Transfer and accumulation of cadmium, and the level of metallothionein in perfused human placentae. Arch Toxicol 65:318–323, 1991.
39. Piasek M, Laskey JW, Kostial K, Blanusa M. Assessment of steroid disruption using cultures of whole ovary and/or placenta in rat and in human placental tissue. Int Arch Occup Environ Health 75(Suppl):S36–S44, 2002.
40. Wier PJ, Miller RK, Maulik D, di Sant' Agnese PA. Toxicity of cadmium in the perfused human placenta. Toxicol Appl Pharmacol 105:156–171, 1990.
41. di Sant' Agnese PA, Jensen KD, Levin A, Miller RK. Placental toxicity of cadmium in the rat: an ultrastructural study. Placenta 4:149–163, 1983.
42. Falcone T, Little AB. Placental synthesis of steroid hormones. In: Tulchinsky D, Ryan KJ, Eds. Maternal-Fetal Endocrinology. Philadelphia: W. B. Saunders Company, pp3–16, 1980.
43. Huszar G. Biology of the myometrium and cervix. In: Warsham J, Ed. The Biological Basis of Reproductive and Developmental Medicine. New York: Elsevier Biomedical, pp85–104, 1983.
44. Trottier B, Athot J, Ricard AC, Lafond J. Maternal-fetal distribution of cadmium in the guinea pig following a low dose inhalation exposure. Toxicol Lett 129:189–197, 2002.
45. Egawa M, Yasuda K, Nakajima T, Okada H, Yoshimura T, Yuri T, Yasuhara M, Nakamoto T, Nagata F, Kanzaki H. Smoking enhances oxytocin-induced rhythmic myometrial contraction. Biol Reprod 68:2274–2280, 2003.
46. Cnattingius S, Granath F, Petersson G, Harlow BL. The influence of gestational age and smoking habits on the risk of subsequent preterm deliveries. N Engl J Med 341:943–948, 1999
47. Hanke W, Kalinka J, Florek E, Sobala W. Passive smoking and pregnancy outcome in central Poland. Hum Exp Toxicol 18:265–271, 1999.
48. Ahern J, Pickett KE, Selvin S, Abrams B. Preterm birth among African American and white women: a multilevel analysis of socioeconomic characteristics and cigarette smoking. J Epidemiol Community Health 57:606–611, 2003.
49. Phung H, Bauman A, Nguyen TV, Young L, Tran M, Hillman K. Risk factors for low birth weight in a socio-economically disadvantaged population: parity, marital status, ethnicity and cigarette smoking. Eur J Epidemiol 18:235–243, 2003.
50. Ventura SJ, Hamilton BE, Mathews TJ, Chandra A. Trends and variations in smoking during pregnancy and low birth weight: evidence from the birth certificate, 1990–2000. Pediatrics 111:1176–1180, 2003.
51. Dejmek J, Solansky I, Podrazilova K, Sram RJ. The exposure of nonsmoking and smoking mothers to environmental tobacco smoke during different gestational phases and fetal growth. Environ Health Perspect 110:601–606, 2002.
52. Miller WL. Molecular biology of steroid hormone synthesis. Endocr Rev 9:295–318, 1988.
53. Ringler G, Strauss JF III. *In vitro* systems for the study of human placental endocrine function. Endocr Rev 11:105–123, 1990.
54. Henson MC, Shi W, Greene SJ, Reggio BC. Effects of pregnant human, nonpregnant human, and fetal bovine sera on human chorionic gonadotropin, estradiol, and progesterone release by cultured human trophoblast cells. Endocrinology 137:2067–2074, 1996.

55. Richards RG, Hartman SM, Handwerger S. Human cytotrophoblast cells cultured in maternal serum progress to a differentiated syncytial phenotype expressing both human chorionic gonadotropin and human placental lactogen. Endocrinology 135:321–329, 1994.
56. Hinkle PM, Osbourne ME. Cadmium toxicity in rat pheochromocytoma cells: studies on the mechanism of uptake. Toxicol App Pharmacol 124:91–98, 1994.
57. Blazka ME, Shaikh ZA. Differences in cadmium and mercury uptakes by hepatocytes: role of calcium channels. Toxicol Appl Pharmacol 110:355–363, 1991.
58. Tang N, Enger D. Cd^{2+}-induced c-*myc* mRNA accumulation in NRK-49F cells is blocked by protein kinase inhibitor H7 but not by HA1004, indicating that protein kinase C is a mediator of the response. Toxicology 81:155–164, 1993.
59. Nishizuka Y. Studies and prospectives of protein kinase C. Science 233:305–312, 1986.
60. Long GJ. The effect of cadmium on cytosolic free calcium, protein kinase C, and collagen synthesis in rat osteosarcoma (ROS17/2.8) cells. Toxicol Appl Pharmacol 143:189–195, 1997.
61. Hechtenberg S, Beyersmann D. Inhibition of sarcoplasmic reticulum Ca^{2+}-ATPase activity by cadmium, lead and mercury. Enzyme 45:109–115, 1991.
62. Misra UK, Gawdi G, Akabani G, Pizzo SV. Cadmium-induced DNA synthesis and cell proliferation in macrophages: the role of intracellular calcium and signal transduction mechanisms. Cell Signal 14:327–340, 2002.
63. Smith JB, Dwyer SD, Smith L. Cadmium evokes inositol polyphosphate formation and calcium mobilization: evidence for a cell surface receptor that cadmium stimulates and zinc antagonizes. J Biol Chem 264:7115–7118, 1989.
64. Jayes FCL, Day RN, Garmey JC, Urban RJ, Zhang G, Veldhuis JD. Calcium ions positively modulate follicle-stimulating hormone- and exogenous cyclic 3′, 5′-adenosine monophosphate-driven transcription of the P450scc gene in porcine granulosa cells. Endocrinology 141:2377–2384, 2000.
65. Matsuoka M, Call KM. Cadmium-induced expression of immediate early genes in LLC-PK_1 cells. Kidney Int 48:383–389, 1995.
66. Hoeffler JP, Meyer TE, Yun Y, Jameson JL, Habener JF. Cyclic AMP-responsive DNA-binding protein: structure based on a cloned placental cDNA. Science 242:1430–1433, 1988.
67. Albright SR, Tjian R. TAFs revisited: more data reveal new twists and confirm old ideas. Gene 242:1–13, 2000.
68. Goyer R. Toxic effects of metals. In: Amdur M, Doull J, Klassen C, Eds. Toxicology: The basic science of poisons. New York: Macmillan, pp623–680, 1991.
69. Mellon SH, Vaisse C. cAMP regulates P450scc gene expression by a cycloheximide-insensitive mechanism in cultured mouse Leydig MA-10 cells. Proc Natl Acad Sci U S A 86:7775–7779, 1989.
70. Brentano ST, Miller WL. Regulation of human cytochrome P450scc and adrenodoxin mRNAs in JEG-3 cytotrophoblast cells. Endocrinology 131:3010–3018, 1992.
71. Ringler GE, Kao L-C, Miller WL, Strauss JF III. Effects of 8-bromo-cAMP on expression of endocrine functions by cultured human trophoblast cells: regulation of specific mRNAs. Mol Cell Endocrinol 61:13–21, 1989.
72. Little B, Billiar RB, Halla M, Heinsons A, Jassani M, Kline IT, Purdy RH. Pregnenolone production in pregnancy. Am J Obstet Gynecol 111:505–514, 1971.
73. Beaudoin C, Blomquist CH, Bonenfant M, Tremblay Y. Expression of the genes for 3β-hydroxysteroid dehydrogenase type 1 and cytochrome P450scc during syncytium formation by human placental cytotrophoblast cells in culture and the regulation by progesterone and estradiol. J Endocrinol 154:379–387, 1997.
74. Orly J, Rei Z, Greenberg NM, Richards JS. Tyrosine kinase inhibitor AG18 arrests FSH-induced granulosa cell differentiation: use of RT-PCR assay for multiple mRNA. Endocrinology 134:2336–2346, 1994.
75. Chung BC, Matteson KJ, Voutilainen R, Mohandas TK, Miller WL. Human cholesterol side-chain cleavage enzyme, P450scc: cDNA cloning, assignment of the gene to chromosome 15, and expression in the placenta. Proc Natl Acad Sci U S A 83:8962–8966, 1986.
76. Inoue H, Higashi Y, Morohashi K, Fujii-Kuriyama Y. The 5′-flanking region of the human P450(SCC) gene shows responsiveness to cAMP-dependent regulation in a transient gene-expression system of Y-1 adrenal tumor cells. Eur J Biochem 171:435–440, 1988.
77. Moore CC, Hum DW, Miller WL. Identification of positive and negative placenta-specific basal elements and a cAMP response element in the human gene for P450scc. Mol Endocrinol 6:2045–2058, 1992.
78. Kostrzewska A, Sobieszek A. Diverse actions of cadmium on the smooth muscle myosin phosphorylation system. FEBS Lett 263:381–384, 1990.
79. Sunderman FW Jr., Barber AM. Finger-loops, oncogenes and metals. Ann Clin Lab Sci 18:267–288, 1988.
80. Vallee BL, Ulmer DD. Biochemical effects of mercury, cadmium and lead. Ann Rev Biochem 41:91–128, 1972.
81. Jacobson KB, Turner JE. The interaction of cadmium and certain other metal ions with proteins and nucleic acids. Toxicology 16:1–37, 1980.
82. Borgeest C, Greenfield C, Tomic D, Flaws JA. The effects of endocrine disrupting chemicals on the ovary. Front Biosci 7:d1941–d1948, 2002.
83. Guillette LJ, Crain DA, Gunderson MP, Kools SAE, Milnes MR, Orlando EF, Rooney AA, Woodward AR. Alligators and endocrine disrupting contaminants: a current perspective. Am Zool 40:438–452, 2002.
84. Garcia-Morales P, Saceda M, Kenney N, Salomon DS, Kim N, Salomon DS, Gottardis MM, Solomon HB, Sholler PF, Jordan VC, Martin MB. Effect of cadmium on estrogen receptor levels and estrogen-induced responses in human breast cancer cells. J Biol Chem 269:16896–16901, 1994.
85. Martin BM, Reiter R, Pham T, Avellanet YR, Camara J, Lahm M, Pentecost E, Pratap K, Gilmore BA, Diverakar S, Dagata RS, Bull JL, Stoica A. Estrogen-like activity of metals in MCF-7 breast cancer cells. Endocrinology 144:2425–2436, 2003.
86. Johnson MD, Kenney N, Stoica A, Hillakivi-Clarke L, Singh B, Chepko G, Clarke R, Sholler PF, Lirio AA, Foss C, Reiter R, Trock B, Paik S, Martin MB. Cadmium mimics the *in vivo* effects of estrogen in the uterus and mammary gland. Nat Med 9:1081–1084, 2003.
87. Stoica A, Katzenellenbogen BS, Martin MB. Activation of estrogen receptor-alpha by the heavy metal cadmium. Mol Endocrinol 14:545–553, 2000.
88. Ng TB, Liu WK. Toxic effect of heavy metals on cells isolated from the rat adrenal and testis. *In Vitro* Cell Dev Biol 26:24–28, 1990.
89. Mgbonyebi OP, Smothers CT, Mrotek JJ. Modulation of adrenal cell functions by cadmium salts: 1. Cadmium chloride effects on basal and ACTH-stimulated steroidogenesis. Cell Bio Toxicol 9:223–234, 1993.
90. Terracio L, Nachtigal M. Oncogenicity of rat prostate cells transformed *in vitro* with cadmium chloride. Arch Toxicol 61: 450–456, 1988.
91. Beyersmann D, Hechtenburg S. Cadmium, gene regulation, and cellular signaling in mammalian cells. Toxicol Appl Pharmacol 144:247–261, 1997.
92. Waalkes MP, Misra RR. In: Chang LW, Ed. Toxicology of Metals. Boca Raton: CRC Press, p 231, 1996.
93. Kondoh M, Araragi S, Sato K, Higashimoto M, Takiguchi M, Sato M. Cadmium induces apoptosis partly via caspase-9 activation in HL-60 cells. Toxicology 170:111–117, 2002.