BIOLOGY OF REPRODUCTION **70,** 25–31 (2004)
Published online before print 3 September 2003.
DOI 10.1095/biolreprod.103.019000

Cadmium Stimulates Transcription of the Cytochrome P450 Side Chain Cleavage Gene in Genetically Modified Stable Porcine Granulosa Cells[1]

Andrea D. Smida,[3] Ximena P. Valderrama,[5] Maria C. Agostini,[6] Michael A. Furlan,[4] and Jorge Chedrese[2,4]

Toxicology Centre,[3] Department of Obstetrics, Gynecology, and Reproductive Sciences,[4] and Department of Veterinary Biomedical Sciences,[5] University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W8
Facultad de Ciencias Veterinarias,[6] UNCPBA, Tandil, Argentina

ABSTRACT

We investigated the effects of cadmium (Cd^{2+}) on transcription of the cytochrome P450 side chain cleavage (P450scc) gene and on progesterone synthesis in stable granulosa cells. We used the stable porcine granulosa cell line, JC-410, genetically modified to express a luciferase genomic construct carrying 2320 base pairs (bp) of the P450scc gene promoter (P450scc-2320-LUC). A construct containing only the luciferase gene, pOLUC, was used as a promoterless control. At 1 μM, cadmium chloride ($CdCl_2$) increased transient expression of P450scc-2320-LUC in JC-410 cells by 2.6-fold after 24-h incubation. A similar pattern of stimulation by $CdCl_2$ was observed in cells transiently transfected with a luciferase genomic construct carrying 100 bp of the P450scc gene promoter P450scc-100-LUC, whereas no stimulation by $CdCl_2$ was observed in cells transfected with pOLUC. At 0.6, 1, and 2 μM, $CdCl_2$ stimulated the activity of the P450scc-2320-LUC promoter in a dose-related fashion by 1.58-, 3.19-, and 2.67-fold, respectively, after 24-h incubation. Northern blot analysis showed that $CdCl_2$ at 0.1, 1, 2, and 3 μM increased P450scc mRNA levels by 3.13-, 1.38-, 1.61-, and 1.57-fold, respectively, after 24-h incubation. After 48-h incubation, $CdCl_2$ at 0.6, 1, and 2 μM further increased P450scc mRNA levels by 3.43-, 2.08-, and 2.4-fold, respectively. At 1, 2, and 3 μM, $CdCl_2$ inhibited progesterone synthesis to 0.48-, 0.38-, and 0.29-fold, respectively. After 48-h incubation, $CdCl_2$ at 0.1 μM stimulated progesterone synthesis by 1.6-fold. We conclude that Cd^{2+} has a dual action in stable porcine granulosa cells: Low concentrations activate, whereas high concentrations inhibit, expression of the P450scc gene and progesterone synthesis. The stimulatory effect of Cd^{2+} appears to be mediated via a *cis*-acting element located 100 bp upstream of the P450scc gene transcription start site.

gene regulation, granulosa cells, progesterone, steroid hormones, toxicology

INTRODUCTION

Cadmium (Cd^{2+}) is a heavy metal that is relatively dispersed in the environment, mainly because of pollution from modern industrial processes, waste disposal, and cigarette smoke [1, 2]. It has no known biological function, and prolonged exposure causes long-term toxic effects to humans and animals [3]. Mainly because of its low rate of excretion from the body, Cd^{2+} has a long biological half-life and accumulates over time in blood, kidney, and liver [1, 4–6] as well as in the reproductive organs, including the placenta, testis, and ovaries [3, 6–9]. Higher concentrations of Cd^{2+} were found in the follicular fluid and in the placentas of smokers, who have reduced levels of progesterone [3, 9]. These observations support the concept that cigarette smoking is an important additional source of Cd^{2+} contamination. Thus, the reproductive organs of smokers are at higher risk of exposure to toxic levels of Cd^{2+}.

The effects of Cd^{2+} on reproduction have been described, but results vary depending on the experimental model and the dose used. In pseudopregnant rats and in cultured rat and human granulosa cells, Cd^{2+} inhibits progesterone synthesis [8, 10, 11]. Although the mechanisms of action on steroidogenesis remain elusive, the results of recent studies conducted in cultured human placental trophoblast cells suggest that Cd^{2+} reduces progesterone synthesis by inhibiting expression of the low-density lipoprotein (LDL) receptor (LDL-R) [12] and the steroidogenic genes, including cytochrome P450 side chain cleavage (P450scc) and 3β-hydoxysteroid dehydrogenase (3β-HSD) [13]. Other reports suggest that Cd^{2+} can stimulate steroidogenesis. When administrated to female rats during estrus and diestrus, Cd^{2+} increases serum progesterone levels [8, 14]. In addition, Cd^{2+} is reported to have stimulatory effects on progesterone synthesis in cultured porcine granulosa cells [5] and in the JAR choriocarcinoma cells, a malignant trophoblast cell line [15].

In the present study, we investigated the effects of Cd^{2+} on expression of the P450scc gene. A hormonally regulated, rate-limiting steroidogenic enzyme, P450scc catalyzes the conversion of cholesterol into pregnenolone [16]. We used a steroidogenically stable porcine granulosa cell line, JC-410 [17], genetically modified with gene constructs containing the promoter region of the P450scc gene linked to a luciferase reporter gene. Using this model, we investigated both the stimulatory and inhibitory effects of Cd^{2+}.

MATERIALS AND METHODS

Reagents

Tissue culture reagents, including phenol red-free medium 199 (M199), penicillin, streptomycin, trypsin, newborn calf serum (NBCS), Lipofectamine 2000, and Geneticin (G-418), were purchased from Gibco (Burlington, ON, Canada). Bovine insulin, luciferin, L-glutamine, cholera toxin, forskolin, 8-bromoadenosine 3′:5′-cyclic monophosphate (8-Br-cAMP), and general chemicals for radioimmunoassay, RNA preparation, and Northern blotting were purchased from Sigma (St. Louis, MO). Plastic

[1]Funded by the Canadian Network of Toxicology Centers, Natural Sciences and Engineering Research Council of Canada, Saskatchewan Agricultural Development Fund, J. Murray Research Grant, and Clinical Teaching and Research Grant, College of Medicine, University of Saskatchewan.
[2]Correspondence: Jorge Chedrese, Department of Obstetrics, Gynecology, and Reproductive Sciences, Royal University Hospital, 103 Hospital Drive, Saskatoon, SK, Canada S7N 0W8. FAX: 306 966 8040; e-mail: chedresj@duke.usask.ca

Received: 5 May 2003.
First decision: 23 May 2003.
Accepted: 4 August 2003.

ISSN: 0006-3363. http://www.biolreprod.org

culture plates were purchased from Falcon (Lincoln Park, NJ). The Bio-Rad DC Protein Assay kit was purchased from Bio-Rad Laboratories (Hercules, CA). Cadmium chloride ($CdCl_2$) was purchased from Aldrich Chemical (Milwaukee, WI). Tracer Amersham IM-140 progesterone glucuronide-^{125}I and nylon membranes were purchased from Amersham (Arlington Heights, IL).

Plasmid Constructs

The porcine P450scc reporter gene constructs, P450scc-2320-LUC and P450scc-100-LUC, and the promoterless pOLUC luciferase reporter vectors were kindly provided by Dr. R.J. Urban (University of Texas, Medical Branch, Galveston, TX). The P450scc-2320-LUC and the P450scc-100-LUC constructs contain 2320 and 100 base pairs (bp), respectively, of the 5′-flanking sequence of the P450scc gene. These sequences were cloned in the plasmid pSVPLUC, which expresses the luciferase gene [18]. The pOLUC vector contains the luciferase cDNA with unique *Sal*I, *Sph*I, *Bam*HI, *Sma*I, and *Hind*III restriction endonuclease sites upstream (5′) to the cDNA translation codon [18]. The plasmid pSV-neo [19], which expresses the neomycin resistance gene, was used to impart resistance to the neomycin analog, G-418, and to select for stably transfected JC-410 cells.

Transfection of JC-410 Granulosa Cells

Cells were cultured in complete culture media consisting of M199 supplemented with 200 mM glutamine, 5% NBCS, 5 μg/ml of insulin, 100 IU/ml of penicillin, and 100 μg/ml of streptomycin as previously described [17]. Cultures were maintained in a CO_2 incubator (Forma Scientific, Inc., Marietta, OH) at 37°C in a water-saturated atmosphere of 95% air and 5% CO_2.

The first group of experiments was conducted in cells transiently transfected with the reporter gene constructs, P450scc-2320-LUC, P450scc-100-LUC, and pOLUC, using the Lipofectamine 2000 protocol. Briefly, cells were cultured in 24-well (1.5- × 2.0-cm) culture plates until attainment of 95% confluency. Media were then replaced with culture media without antibiotics (transfection media) but with 1 μg of plasmid DNA. Twenty-four hours after transfection, 1 μM $CdCl_2$ was added for 24 h. Experiments were terminated by collecting cells with extraction buffer (1% Triton X-100 and 1 mM dithiothreitol [DTT]) and assayed for luciferase activity.

The second group of experiments was conducted in cells stably transfected with the P450scc-2320-LUC. Cells were transfected using the Lipofectamine 2000 protocol with 0.85 μg of the P450scc-2320-LUC plasmid and 0.15 μg of the pSVneo plasmid (5:1 ratio). A separate culture was transfected with 1 μg of the P450scc-2320-LUC plasmid as a transfection control group. Twenty-four hours after transfection, cells were removed with trypsin and replated at 1:10 dilution into 35 mm (24-well) culture plates. After 24-h culture, media were replaced with complete M199 containing 0.4 mg/ml of the antibiotic G-418. Cells were then cultured for 2 to 3 wk until visible colonies (≥1000 cells) were observed. Colonies were removed from the plates using sterile glass cloning cylinders according to the method described by Southern and Berg [19]. Colonies were harvested after the addition of 50 μl of trypsin for 2 min at 37°C. Colonies were then transferred into 24-well plates and maintained with complete culture media containing 0.133 mg/ml of G418. Once colonies reached 80–95% confluency, they were transferred into 35 mm well plates. Each colony was selected by its ability to express luciferase activity. Colonies that were deemed to be positive underwent further characterization to known stimulators of the protein kinase A pathway, such as cholera toxin, forskolin, and 8-Br-cAMP. The colony that provided the greatest sensitivity to these stimulators was used for further experiments with $CdCl_2$. Experiments were conducted by adding $CdCl_2$ for the indicated concentrations and times, and they were terminated by collecting cells and/or media for the respective assay.

Luciferase Assay

Luciferase assay was carried out as described by Chedrese et al. [20]. Cells were harvested by adding 200 μl/well of extraction buffer and centrifuged for 5 min at 4°C (12 000 × *g*). Luciferase activity was measured in 100 μl of the supernatant diluted in 368 μl of luciferase buffer (25 mM gly-glycine, 15 mM magnesium sulfate, 4 mM EGTA, 16 mM potassium phosphate, 1 mM DTT, and 2 mM ATP; pH 7.8) at room temperature. Light emission was determined using a Monolight 2010 luminometer (Analytical Luminescence Laboratory, San Diego, CA), which injects 100 μl of 25 mM luciferin in glyclglycine buffer (25 mM glyclglycine, 15 mM magnesium sulfate, 4 mM EGTA, and 10 mM DTT; pH 7.8) into the cell lysate. Luciferase activity was measured by calculating the light emitted during the initial 10 sec of the reaction, and the values were expressed as relative light units. The results were then normalized by dividing the values for each treatment by the respective control values and expressed as fold-increases or fold-decreases compared to the mean control level.

Progesterone and Protein Assays

Progesterone concentration was determined by radioimmunoassay in 100 μl of culture media as previously described by Rawlings et al. [21]. The inter- and intraassay coefficients of variation for the progesterone assay were less than 10%, and the minimum detectable content was 6.35 pg. Total protein content was determined in cells collected with 250 μl of 0.1% SDS using the Bio-Rad DC Protein Assay kit. Progesterone synthesis was normalized between experiments by expressing progesterone levels divided by the total cellular protein content per well.

Northern Blot Analysis

Total RNA was isolated by acid phenol:chloroform extraction according to the method described by Chomezynski and Sacchi [22]. Samples of total RNA were denatured, size-fractionated by electrophoresis on 1% agarose-formaldehyde gel, and transferred onto a nylon membrane by diffusion blotting. The RNA was cross-linked to the membrane using a ultraviolet Stratalinker 1800 (Stratagene, La Jolla, CA). Porcine P450scc [23] and mouse glyceraldehyde-3-phosphate dehydrogenase [24] cDNAs were used as probes. Probes were labeled by primer extension [25] with [α-^{32}P]dCTP (>3000 Ci mmol^{-1}; New England Nuclear, Boston, MA) to a specific activity of 1.5–3.0 × 10^9 dpm mg^{-1} DNA. Membranes were hybridized using the Quik Hyb hybridization solution according to the manufacturer's instructions (Stratagene) and autoradiographed. A Kodak Electrophoresis Documentation and Analysis System 120 (Eastman Kodak, Rochester, NY) was used for gel photography and densitometric analysis of autoradiographs.

Statistical Analyses

Data are presented as the mean ± SEM of three independent experiments. Data were subjected to one-way ANOVA. The Fisher least significant difference test was used for comparisons of individual means (Student Edition of Statistix, Windows Version 2.0, 1998, Analytical Software, Tallahassee, FL). Significant differences were established at $P \leq 0.05$.

RESULTS

The effect of $CdCl_2$ on the JC-410 cells transiently transfected with P450scc genomic constructs is shown in Figure 1. At 1 μM, $CdCl_2$ increased by 2.6- and 3.3-fold the luciferase activity of cells transfected with the construct P450scc-100-LUC and P450scc-2320-LUC, respectively, after 24-h incubation. No effect was observed in cells transiently transfected with pOLUC.

The luciferase activity and protein content of JC-410 cells genetically modified to stably express P450scc-2320-LUC and treated for 24 h with $CdCl_2$ are shown in Figure 2. At 0.6, 1, and 2 μM, $CdCl_2$ stimulated cellular luciferase activity by 1.6-, 3.2-, and 2.7-fold, respectively (Fig. 2A). Incubation of cells with 3 and 5 μM $CdCl_2$ reduced activity to 0.9- and 0.5-fold, respectively (Fig. 2A). The total protein content was not affected in cells treated with $CdCl_2$ at concentrations of up to 3 μM (Fig. 2B). However, $CdCl_2$ at 5 μM reduced total cellular protein content to 0.54-fold over control (54 vs. 29 mg/well) (Fig. 2B) as well as cell number (data not shown).

The effect of $CdCl_2$ on cell morphology is shown in Figure 3. Untreated cells contact each other, showing extensive cellular extensions or pseudopodia (Fig. 3A). No morphological changes were observed in cells exposed to 1 μM $CdCl_2$ (Fig. 3B). However, when cells were exposed to 5 μM $CdCl_2$, they exhibited rounded shapes and contraction of cellular extensions (Fig. 3C). Fragmented cytoplasmic processes (remainders of pseudopodia) were observed attached to growing surfaces of the contracted cells.

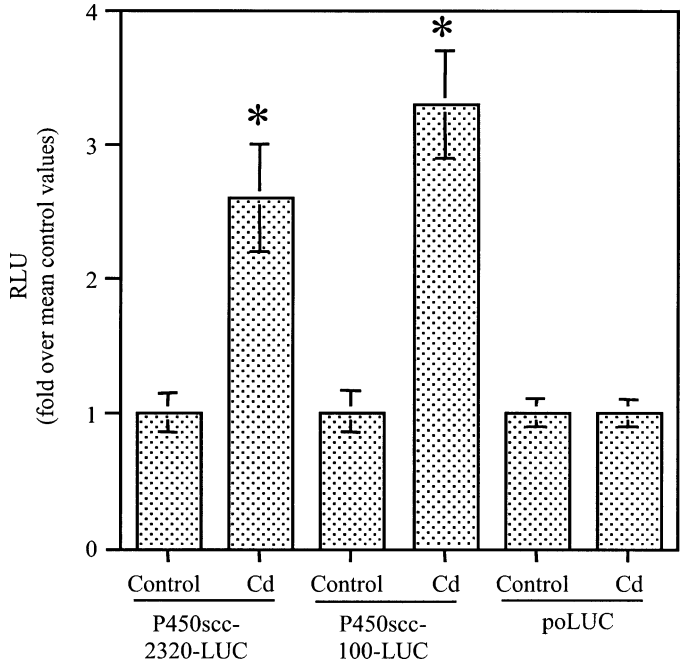

FIG. 1. Effect of $CdCl_2$ (Cd) on JC-410 cells transiently transfected with P450scc-100-LUC, P450scc-2320-LUC, and poLUC. Cells were transiently transfected as described in *Materials and Methods* and exposed to 1 μM $CdCl_2$. After 24-h incubation, cells were collected and assayed for luciferase activity. Data, expressed as relative light units (RLU) over the mean control value for each treatment, were analyzed by one-way ANOVA followed by the Fisher least significant difference test. Each bar represents the mean ± SEM of three independent replications. $^*P < 0.05$.

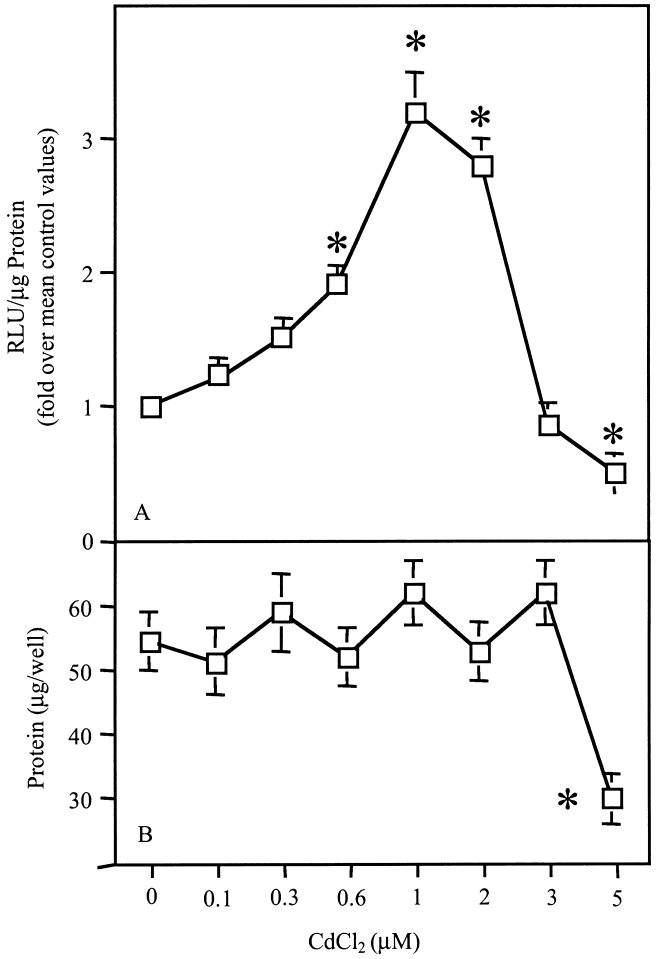

FIG. 2. Effect of $CdCl_2$ on JC-410 cells stably transfected with P450scc-2320-LUC. Cells were exposed to the indicated concentrations of $CdCl_2$. After 24-h incubation, cells were collected and assayed for luciferase activity. Data regarding relative light units (RLU) divided by total protein content were expressed as a fold-change over the mean control value for each treatment and analyzed by one-way ANOVA followed by the Fisher least significant difference test. Each point represents the mean ± SEM of three independent replications. $^*P < 0.05$.

The effect of time of exposure to $CdCl_2$ on luciferase activity of JC-410 calls stably transfected with the P450scc-2320-LUC is shown in Figure 4. At 1 μM, $CdCl_2$ increased luciferase activity by 1.77-, 3.6-, and 5.6-fold after 18-, 24-, and 48-h incubation, respectively.

The effect of concentration and time of exposure of $CdCl_2$ to JC-410 cells on progesterone synthesis is shown in Figure 5. A numeric, but not significant, increase in progesterone synthesis was observed after 24-h incubation with 0.1 μM $CdCl_2$. At 0.6, 1, 2, and 3 μM, $CdCl_2$ decreased progesterone synthesis by 0.7-, 0.4-, 0.38-, and 0.29-fold, respectively. After 48-h incubation, 0.1 μM $CdCl_2$ stimulated progesterone synthesis by 1.62-fold, but at 0.6, 1, 2, and 3 μM, no changes in progesterone synthesis were observed.

The effect of $CdCl_2$ on P450scc mRNA levels in JC-410 cells is shown in Figure 6. Cells responded to 100 ng/ml of cholera toxin with a 5.3- and 3.1-fold increase in the P450scc mRNA levels after 24- and 48-h incubation, respectively. After 24-h incubation, $CdCl_2$ at 0.6, 1, 2, and 3 μM stimulated P450scc mRNA levels by 3.1-, 1.4-, and 1.6-folds, respectively. After 48-h incubation, 0.6, 1, and 2 μM $CdCl_2$ stimulated P450scc mRNA levels by 3.4-, 2.1-, and 2.4-fold, respectively. However, a 0.5-fold decrease in the P450scc mRNA levels was observed when cells were incubated with 3 μM $CdCl_2$ for a 48-h period. The same patterns of response to $CdCl_2$ observed in P450scc mRNA levels were seen during three independent replications of the same experiment.

DISCUSSION

We established a genetically modified, porcine, stable granulosa cell line to study the effects of $CdCl_2$ on expression of the P450scc gene. We observed that low (0.6–3 μM) and high (5 μM) concentrations of $CdCl_2$ in the culture media have opposite effects on P450scc promoter activity. At low concentrations, $CdCl_2$ stimulates P450scc gene promoter activity in a dose- and time-dependent fashion, whereas at high concentrations, it inhibits 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 reduced cell number, reduced cellular protein content, and altered cell morphology. Overall, this information suggests that Cd^{2+} has a dual action in the granulosa cells: Low concentrations activate, whereas high concentrations inhibit, expression of the P450scc gene.

The mechanisms by which Cd^{2+} affects cell function and gene expression were recently reviewed by Bhattacharyya et al. [1]. It has been shown that Cd^{2+} can easily enter into the cells through the L-type voltage Ca^{2+} channels [26] and receptor-mediated Ca^{2+} channels [27], because both cations have similar radii size and charge (Ca^{2+} = 0.97 Å, Cd^{2+} = 0.99 Å). In addition, Cd^{2+} can displace Ca^{2+} from its

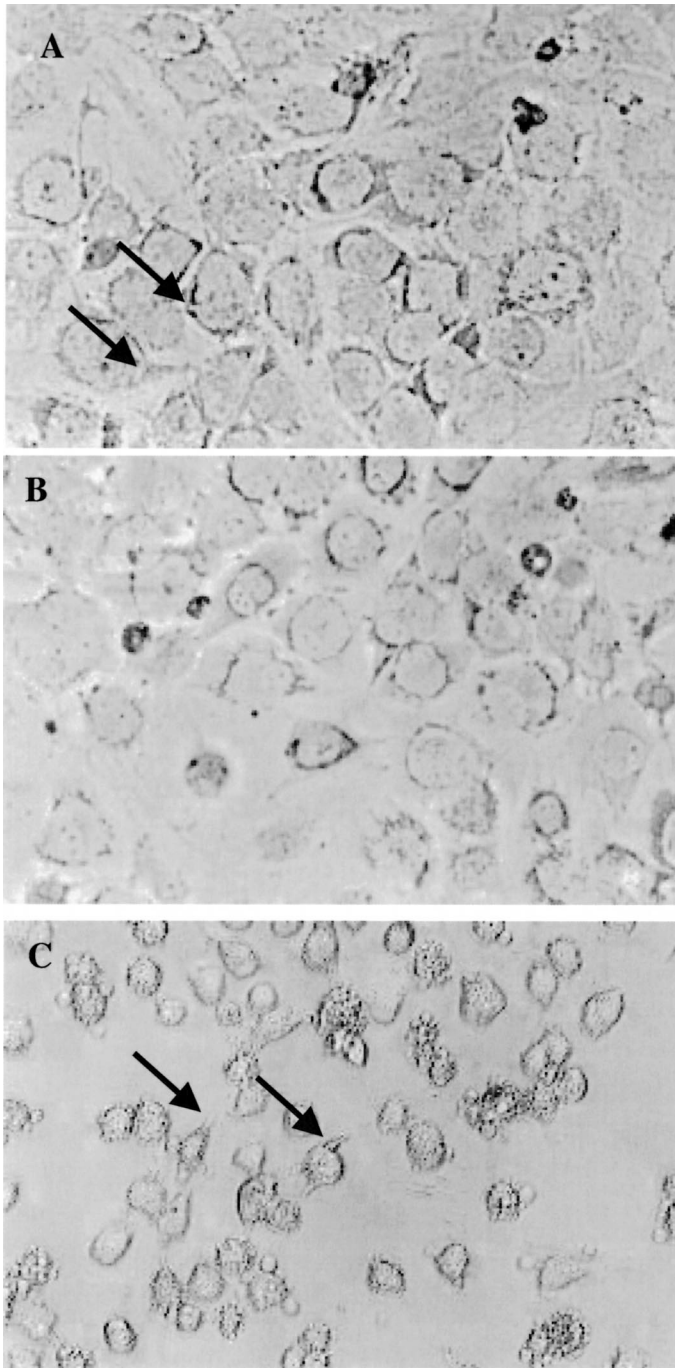

FIG. 3. Effect of $CdCl_2$ on JC-410 cell morphology. Cells were exposed to (**A**) control media, (**B**) 1 μM $CdCl_2$, or (**C**) 5 μM $CdCl_2$ for 24 h. In **A**, arrows indicate areas of contact and pseudopodia. In **C**, arrows indicate remainders of pseudopodia on growing surfaces of contracted cells. Magnification ×60.

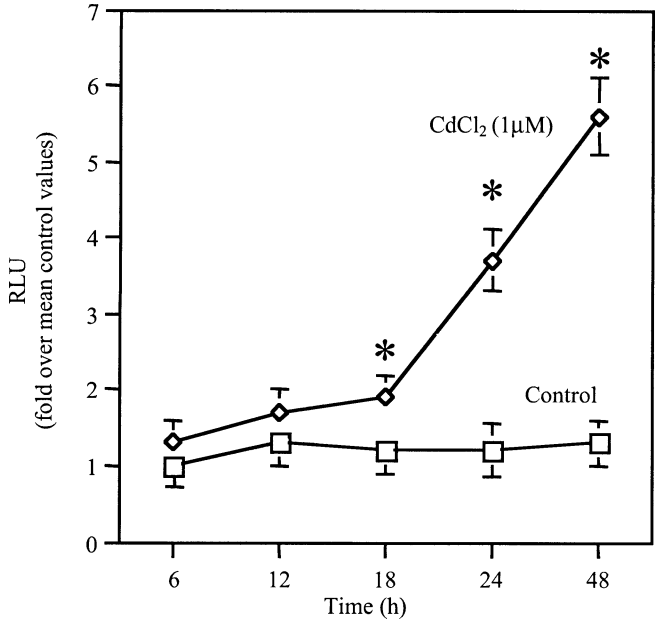

FIG. 4. Effect of time exposure to $CdCl_2$. The JC-410 cells stably transfected with P450scc-2320-LUC were exposed to 1μM $CdCl_2$ for the indicated times, at the end of which they were collected and assayed for luciferase activity. Data regarding relative light units (RLU) were expressed as a fold-change over the mean control value for each treatment and analyzed by one-way ANOVA followed by the Fisher least significant difference test. Each point represents the mean ± SEM of three independent replications. $*P < 0.05$.

normal binding to calmodulin and protein kinase C (PKC) [28]. 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 [28]. The PKC is a family of Ser/Thr kinases, the activation of which is Ca^{2+}- and phospholipid-dependent [29]. Long [30] showed that Cd^{2+} can activate PKC directly with a constant 5000-fold smaller than that of Ca^{2+}. Furthermore, Cd^{2+} at concentrations between 0.1 and 5 μM interferes with the Ca^{2+}-ATPase pumps, leading to an immediate, transient, but substantial increase in intracellular Ca^{2+} [31, 32]. This increase in Ca^{2+} results in the formation of inositol triphosphate, which triggers the PKC signal cascade [33]. 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 [34]. Therefore, it is reasonable to speculate that the low concentrations of Cd^{2+} used in the present study may be sufficient to mimic the effects of Ca^{2+}, resulting in stimulation of the steroidogenic pathway and synthesis of progesterone in the JC-410 cells.

Although Cd^{2+} affects placental and ovarian steroidogenesis, its mechanisms of action have not been clarified, and different studies have provided conflicting information. In cultured human placental trophoblast cells, $CdCl_2$ at concentrations between 5 and 20 μM inhibited expression of the steroidogenic genes, including the LDL-R [4, 12, 35], P450scc, and 3β-HSD [13]. Serum LDL is a major source of cholesterol for progesterone synthesis [36]. Therefore, limited availability of cholesterol and inhibition of the steroidogenic genes could be the cause by which high levels of Cd^{2+} reduce progesterone synthesis, as described in the pseudopregnant rat model [10] and in cultured rat and human granulosa cells [8, 11]. Our studies were conducted in serum-free culture conditions, in which $CdCl_2$ stimulated expression of the P450scc gene and progesterone synthesis. Synthesis of progesterone in the JC-410 stable granulosa cells is entirely dependent on an intracellular source of cholesterol [37]. Therefore, it is reasonable to speculate that the observed effects of Cd^{2+} are independent of the regulation of LDL-R or LDL metabolism. This is novel information that may explain why Cd^{2+} also elevates progesterone levels, as reported in estrous and diestrous rats [8, 14], in cultured porcine granulosa cells [5], and in the JAR choriocarcinoma cells [15].

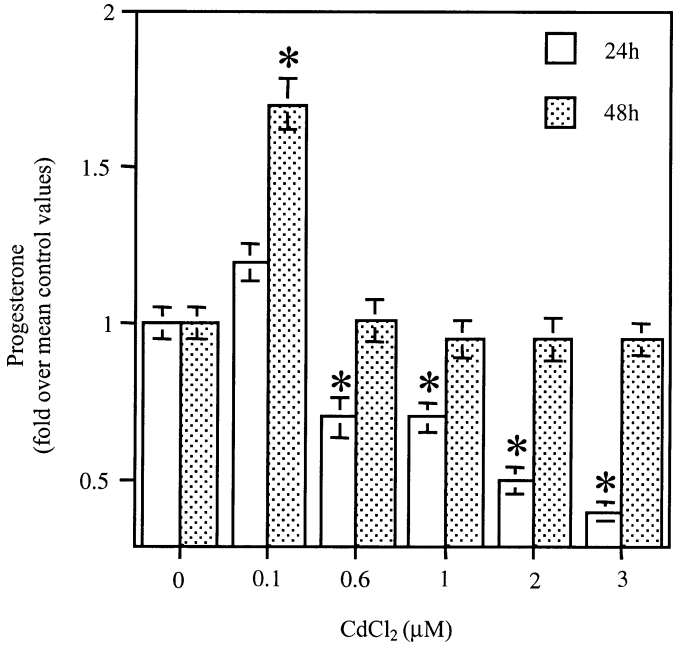

FIG. 5. Effect of $CdCl_2$ on progesterone synthesis. The JC-410 cells were exposed to increased concentrations of $CdCl_2$. At the end of the indicated times, cells and media were collected for protein and progesterone assays, respectively. Progesterone levels were divided by the protein content and expressed as a fold-change over the mean control value for each treatment. Data were analyzed by one-way ANOVA followed by the Fisher least significant difference test. Each bar represents the mean ± SEM of three independent replications. $^*P < 0.05$.

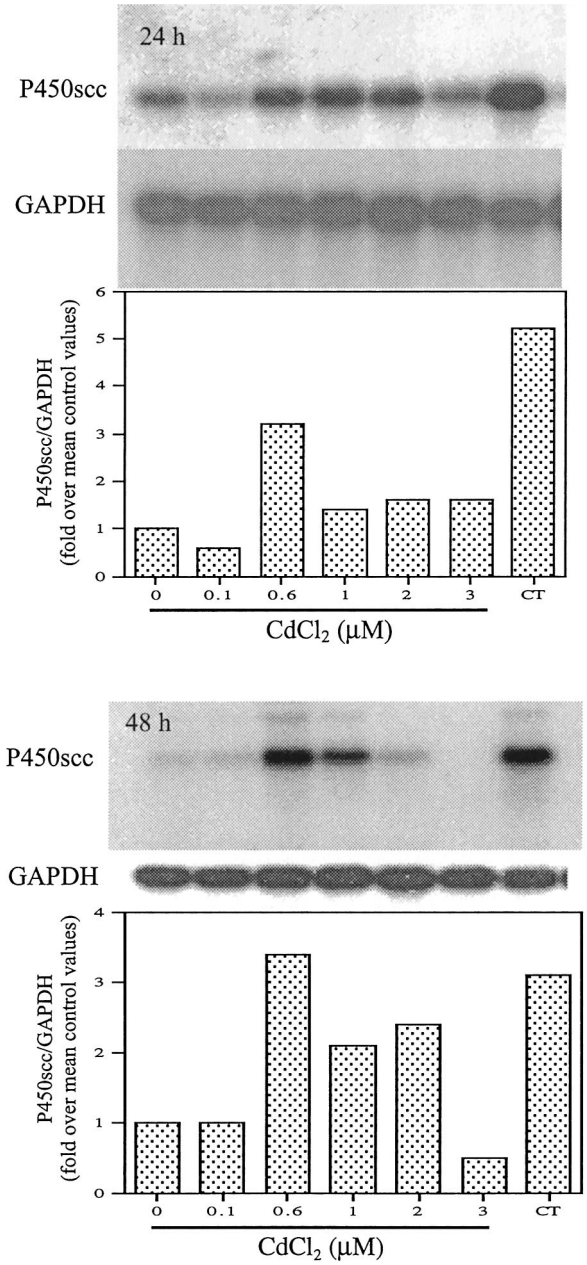

FIG. 6. Effect of $CdCl_2$ on P450scc mRNA levels in JC-410 cells. Cells were exposed for 24 and 48 h to increased concentrations of $CdCl_2$. Cells were also exposed to 100 ng/ml of cholera toxin (CT) as a positive control. At the end of the indicated times, cells were collected for total RNA extraction and examined by Northern blot analysis using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression as a control for RNA loading. Data are from one representative experiment, which was repeated three times. Bottom panels are graphic representations of the densitometric analysis of the autoradiograms.

Overall, our studies support the concept that depending on the concentration, Cd^{2+} can exert dual effects on steroidogenesis. Dual effects of Cd^{2+} have been described in other experimental models as well. At low doses, Cd^{2+} stimulates DNA synthesis, cell multiplication, and malignant transformation [38, 39]. When administered in the millimole range, Cd^{2+} is toxic and is associated with diminished DNA synthesis, apoptosis, and chromosome aberrations [37, 40]. When used in concentrations greater than 2–5 μM, Cd^{2+} induces apoptosis, partially via activation of caspase-9 in HL-60 cells [41]. Therefore, the reduction in cell number, reduction in cellular protein content, and changes in cell morphology observed in the present study possibly resulted from the apoptotic effects of high concentrations of $CdCl_2$.

In the present study, $CdCl_2$ did not change luciferase activity in cells transiently transfected with the promoterless pOLUC, suggesting that the stimulatory effect of $CdCl_2$ is specific to the P450scc gene promoter and is not a general effect on transcription. However, $CdCl_2$ also stimulated activity of P450scc-100-LUC and P450scc-2320-LUC with similar magnitudes (Fig. 1). These observations suggest that a *cis*-acting element located 100 bp upstream of the transcription start site may be involved in $CdCl_2$-stimulated transcription of the P450scc gene. Several sequence motifs with high homology for transcription factors are present in the first 100 bp upstream of the transcription start site of the porcine P450scc gene [18, 42]. They include consensus sequence motifs for the proto-oncogenes c-*jun*, and c-*fos*, the cAMP regulatory element-binding protein, 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 Cd^{2+} induces transcription of c-*jun* and c-*fos* through mechanisms mediated by PKC [43, 44]. On stimulation, c-*jun* and c-*fos* form heterodimers that bind to AP-1 sites in several PKC-activated genes [45]. 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 in its promoter. A zinc-finger transcription factor, Sp1 was originally defined in the promoter of the simian virus SV40 [46]. It 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 [37]. Further investigation needs to be conducted to determine the mechanisms by which transcription factors mediate the effects of Cd^{2+} on the P450scc gene.

Environmental pollution with Cd^{2+} has increased dramatically over the last several decades as a result of industrial processes, including mining, smelting, and electroplating, and the intensive use of other products containing associated metals, such as nickel/cadmium batteries, pigments, and plastics [39]. Thus, Cd^{2+} accumulated in the environment is detectable in meat, fish, and fruits [39]. It is estimated that the daily human intake of Cd^{2+} is between 10 and 40 μg [47]. The Cd^{2+} has a low rate of excretion from the body and accumulates over time [1, 4–6]. Occupational exposure and cigarette smoke are important sources of Cd^{2+} contamination [3]. The results of clinical and experimental observations suggest that elevated concentrations of Cd^{2+} are at least one cause of the reduced levels of progesterone seen in the placenta of smokers [3, 9, 12, 13]. The effects observed in the present study were at concentrations of Cd^{2+} lower than those concentrations (5–20 μM) used to induce endocrine disruption in cultured human trophoblasts [13]. Therefore, it is feasible that the concentrations we used may be representative of the doses at which humans and animals are regularly exposed. Based on our results, it is reasonable to think that even exposure to low concentrations of Cd^{2+} may be sufficient to affect the steroidogenic pathway.

We conclude that the established, genetically modified, stable granulosa cell is an adequate model to study the dual effect of Cd^{2+}. At low concentrations, Cd^{2+} stimulates transcription of the P450scc gene and the steroidogenic pathway. At high concentrations, Cd^{2+} inhibits activity of the P450scc gene and progesterone synthesis, and it has cytotoxic effects that produce 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 a necessary step in the steroidogenic pathway. Therefore, the changes induced by Cd^{2+} on the expression of the P450scc gene in the granulosa cells could affect the synthesis of all steroid hormones in the ovary.

ACKNOWLEDGMENTS

We thank Dr. Randall Urban from the University of Texas, Medical Branch, for providing us with the DNA constructs used in the present study. We also thank Christine Meaden for editorial assistance and critical review of the manuscript.

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