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Altered Cyclic AMP-Dependent Human Chorionic Gonadotropin Production in Cultured Human Placental Trophoblasts Exposed to Ethanol

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ABSTRACT. Chronic ethanol abuse during pregnancy can cause fetal injury, including the fetal alcohol syndrome (FAS). A contributing factor in this fetal injury may be the effect of ethanol on placental function. Previous studies have shown that ethanol treatment increases human chorionic gonadotropin (hCG) production by cultured human placental trophoblasts. In this study, we demonstrated that the stimulation of hCG production correlates with the ethanol concentration. Ethanol treatment enhanced intracellular adenosine 3′:5′-cyclic monophosphate (cAMP) levels in response to either cholera toxin (CTX) or forskolin (FSK). Moreover, basal (i.e. unstimulated) cAMP levels were increased at 2 hr of ethanol exposure. However, this effect did not persist throughout the 24-hr incubation period. Therefore, ethanol treatment appears to induce increased hCG production, secondary to enhanced basal or stimulated cAMP production. The effect of ethanol was not associated with changes in G_s or G_{i2} expression, as determined by northern blot and western blot analyses. In plasma membrane preparations from ethanol-treated cells, cAMP production was higher in response to Mn^{2+}, a direct stimulator of adenylyl cyclase. Inclusion of Rp-cAMP, a protein kinase A inhibitor, eliminated the ethanol effect on hCG production. Treatment of cells with 8-Br-cAMP stimulated hCG production, but there was no difference between the ethanol-naive control and the ethanol-treated cells. These data suggest that ethanol treatment increases *in vitro* hCG production in human placental trophoblasts by enhancing cAMP production. Ethanol treatment appears to increase trophoblast adenylyl cyclase activity. BIOCHEM PHARMACOL 55;1:45–51, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. ethanol; adenylyl cyclase; placenta; trophoblast; adenosine 3′:5′ cyclic monophosphate; human chorionic gonadotropin

Chronic ethanol abuse during pregnancy is associated with fetotoxicity and can lead to the development of the fetal alcohol syndrome (FAS) [1, 2]. Although the mechanisms of ethanol toxicity are not fully characterized, both direct and indirect actions on the fetus may be involved. One indirect mechanism may involve altered placental function. Many placental functions are critical for normal fetal growth and development. Previous studies in this and other laboratories suggest that ethanol may adversely affect placental nutrient transport [3–5]. In cultured human term placental trophoblasts, long-term treatment with ethanol inhibited hormone-stimulated amino acid uptake [4]. In addition, long-term treatment with ethanol enhanced the production of hCG¶ and progesterone [6]. Synthesis of hCG and progesterone is known to be responsive to cAMP [7]. A recent report by this laboratory demonstrated that ethanol causes heterologous sensitization (up-regulation) of the hCG response in trophoblasts exposed to ligands that stimulate cAMP production [8]. Therefore, ethanol enhancement of hCG production may be mediated, in part, by increased cAMP production in trophoblasts. In this study, we examined the effect of various modulators of cAMP production on cAMP and hCG production in ethanol-treated trophoblasts and further defined those signaling components affected by ethanol.

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¶ *Abbreviations:* hCG, human chorionic gonadotropin; cAMP, adenosine 3′:5′-cyclic monophosphate; 8-Br-cAMP, 8-bromoadenosine 3′:5′-cyclic monophosphate; CTX, cholera toxin; FSK, forskolin; Rp-cAMP, adenosine 3′:5′-cyclic phosphorothioate-Rp; DMEM, Dulbecco's Modified Eagle's Medium; FBS, fetal bovine serum; PKA, protein kinase A; and PCA, perchloric acid.

MATERIALS AND METHODS

Materials

DMEM, FBS, and calcium-magnesium (Ca^{2+}-Mg^{2+})-free Hanks' buffer were obtained from Biofluids (Rockville, MD). Dispase was purchased from Collaborative Research (Bedford, MA). Assay kits were obtained as follows: cAMP from either Diagnostic Products Corp. (Los Angeles, CA) or Amersham (Arlington Heights, IL); and hCG from Ciba–Corning Diagnostics (Medfield, MA). Rp-cAMP was from Biomol Research Laboratories, Inc. (Plymouth Meeting, PA). Percoll, 8-Br-cAMP, CTX, FSK, alumina and routine chemicals were purchased from the Sigma Chemical Co. (St. Louis, MO). [α-^{32}P]Adenosine 5′-triphosphate and [^{3}H]-cAMP were from DuPont-NEN Research Products (Boston, MA).

Primary antisera to specific G protein subunits were raised in the rabbit against synthetic peptides corresponding to defined regions of G protein subunits as described previously [11]. Antiserum C584, which recognizes the 45-kDa and 52-kDa forms of $G_s\alpha$, was raised against a synthetic carboxyterminal peptide specific for $G_s\alpha$. Antiserum A54, which recognizes $G_{i2}\alpha$, was raised against a synthetic peptide unique to the middle of $G_{i2}\alpha$ protein and recognizes a 40-kDa protein.

Trophoblast Culture

Normal term placentas were obtained within 15 min of either vaginal or cesarean delivery and processed immediately for trophoblast isolation as modified previously [9, 10]. Purified cytotrophoblasts were diluted in DMEM containing 4 mM glutamine, 50 μg/mL gentamicin, and 20% heat-inactivated FBS. The cells were plated at a density of 1.5×10^5 cells/cm^2 into Falcon Primaria® culture wells, either 35-mm dishes (1.5 mL medium) or 24-well cluster plates (0.5 mL) (Becton Dickinson, Lincoln Park, NJ), and incubated in humidified 5% CO_2 at 37°. The medium was changed every 24 hr.

At the time of harvest, the medium was centrifuged to remove debris, and the supernatant was stored at −20° until assayed for hCG. The cells were washed twice with cold PBS. The washed cells were digested in 0.2 N NaOH/0.2% sodium dodecyl sulfate solution, and the digest was stored at −20° until assayed for protein.

Chronic Ethanol Treatment

Chronic ethanol treatment [6] used a sealed chamber (Modular Incubator Chamber, Billups-Rothenberg, Del Mar, CA, or Desiccator Cabinet, Bel-Art Products, Pequannock, NJ) equilibrated with a 5% CO_2:95% air mixture. Briefly, an ethanol solution was placed in a tray at the bottom of the chamber. Incubation at 37° resulted in vaporization of ethanol, which gradually diffused into the culture medium. Control cells were also maintained under similar conditions except that H_2O was added to the reservoir tray. The ethanol concentration in the medium was determined by the alcohol dehydrogenase method (Behring Diagnostics, Somerville, NJ). The culture medium was refreshed at 24-hr intervals. The ethanol solution in the reservoir was also changed at 24-hr intervals.

Treatment of Trophoblasts with Modulators of cAMP Production and Protein Kinase A

To determine the effect of various modulators of cAMP production and PKA on hCG production in ethanol-treated cells, trophoblasts were exposed to ethanol for 72 hr (300 mg/dL or 65 mM daily peak medium concentration). During the final 20 hr, ethanol-naive control cells and ethanol-exposed cells were treated with CTX (100 ng/mL) and FSK (20 μM), activators of adenylyl cyclase; membrane-permeable cAMP analog 8-Br-cAMP (2 mM); or Rp-cAMP (30 μM), a specific inhibitor of PKA. The culture medium and cells were harvested for hCG and protein assays. For determination of cAMP, CTX- and FSK-treated cells were washed with PBS, extracted with cold 0.5 M PCA for 30 min, neutralized with 25% KOH/30% $KHCO_3$, and assayed for cAMP using a competition binding assay as described previously [8]. Briefly, following removal of $KClO_4$, 150 μL of the supernatant was assayed via a kit for cAMP content, using a competition binding assay (Diagnostic Products Corp.). The cAMP extraction procedure was a modification of a method provided by the manufacturer. The extraction efficiency of this procedure was >94% based upon the recovery data from samples spiked with [^{3}H]cAMP. In preliminary studies, using known amounts of cAMP, PCA extraction did not affect the ligand displacement in the competition binding assay. The assay range of the kit was from 0.11 to 27 pmol/tube. In most experiments, cAMP concentration in the samples ranged from 0.5 to 10 pmol/tube.

Membrane Preparation and Adenylyl Cyclase Assay

Trophoblasts were cultured in 60-mm dishes for 48 hr, with or without ethanol exposure. The cells were washed with cold PBS and scraped into homogenization buffer containing 50 mM Tris–HCl, pH 7.4, 1 mM EDTA, 1 mM dithiothreitol, 0.25 M sucrose, 50 μg/mL leupeptin, and 25 μg/mL aprotinin. The cells were homogenized using a Dounce homogenizer with a tight-fitting pestle (80 strokes) and centrifuged (200 *g* for 5 min) to remove unbroken cell debris and nuclei. The post-nuclear fraction was centrifuged at 110,000 *g* for 30 min to obtain a crude membrane pellet that was resuspended in 50 mM Tris–HCl, pH 7.4, containing 0.25 M sucrose. Adenylyl cyclase activity was determined using a modification of methods described by Alvarez and Daniels [11]. Briefly, the reaction mixture (100 μL) contained membrane protein (20–30 μg), 40 mM Tris–HCl, pH 7.4, 2 mM cAMP, 5 μg BSA, 5 U creatine kinase, 5 mM phosphocreatine, and 1 μCi [α-^{32}P]ATP (final concentration 500 μM), and was incubated at 37° for

15 min. When used, activators of adenylyl cyclase, 20 mM Mn^{2+} or 20 μM FSK, were added to the reaction mixture. The reaction was terminated by the addition of 20 μL of 2.2 M HCl, the mixture was boiled for 4 min, and the sample was chromatographed on neutral alumina as described by Alvarez and Daniels [11]. [^{32}P]cAMP was eluted with 0.1 M ammonium acetate and countered for radioactivity. Using [^{3}H]cAMP, recovery of authentic cAMP was found to be at least 95%. In all experiments, background activity was determined by adding HCl to the reaction mixture prior to the addition of [^{32}P]ATP. This value was corrected prior to calculation of specific activity of adenylyl cyclase.

Western and Northern Blot Analyses for G Proteins, $G_s\alpha$ and $G_{i2}\alpha$

To determine G protein concentration and expression, cells were exposed to ethanol for 48 hr. Cells were scraped into the homogenization buffer and processed as described above to obtain the membrane fraction. Protein samples (50 μg) were solubilized in Laemmli sample buffer and subjected to SDS–PAGE. Separated protein samples were transferred to a nitrocellulose membrane using Towbin transfer buffer. Antipeptide antibody was diluted to obtain optimal saturating concentrations (as determined by preliminary studies) and incubated with the membrane for 16 hr at 4°. The blots were washed and then incubated with optimal concentrations (determined for each lot) of the horseradish peroxidase conjugated antibodies for detection of immunoreactive bands by chemiluminescence (Renaissance, DuPont New England Nuclear). To control for loading variability, each sample was resolved in at least two gels. The X-ray films were exposed for various periods of time to obtain appropriate density of non-overlapping grains. For each film, specific bands were quantified by densitometric scanning of the X-ray film and analysis with ImageQuant software.

To extract total RNA, trophoblasts were scraped into 4 M guanidine thiocyanate, 26 mM trisodium citrate, 0.5 M *n*-lauroyl sarcosine and homogenized using a Dounce homogenizer. RNA was isolated, resolved, and transferred by northern blot to a Duralon-UV membrane (Stratagene). The filters were UV-cross-linked, prehybridized, and then hybridized overnight at 55° with radiolabeled cDNAs. The cDNAs used included a 1.5 kb *NcoI-SalI* cDNA fragment containing the coding sequences and 3′-untranslated sequences for human $G_s\alpha$ and a full-length cDNA corresponding to rat $G_{i2}\alpha$. The cDNA fragments were excised from plasmid DNAs by restriction endonuclease digestion and radiolabeled by random primer extension using [^{32}P]dCTP.

Biochemical Analyses

The hCG in the culture medium was determined using a radioimmunoassay kit (Ciba Corning Diagnostics Corp.) that quantitates both the β subunit and intact hCG.

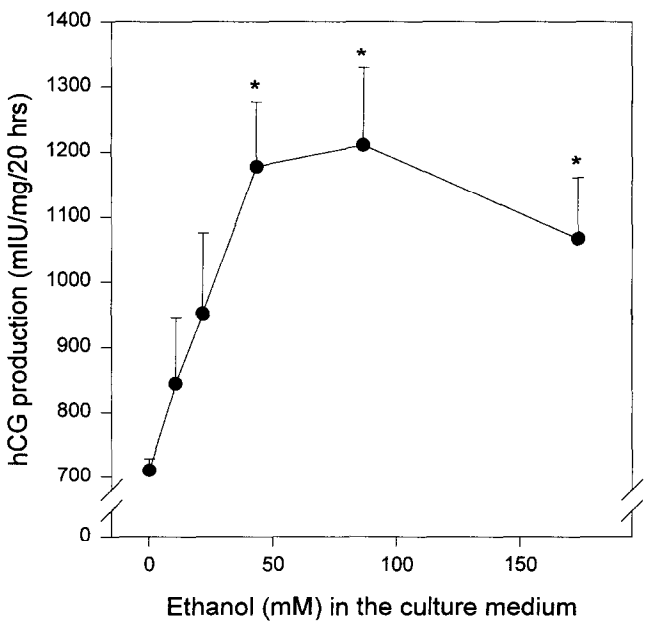

FIG. 1. Effect of ethanol on hCG production. Cultured trophoblasts were placed in Modular incubators with either various concentrations of ethanol or H_2O in the reservoir (50 mL). All solutions were changed daily for 3 days. Following incubation, both the medium and cells were harvested and processed as described in the text. The amount of hCG produced was normalized by total cell protein concentration, and the data were plotted against the final ethanol concentration in the culture medium (N = 4). Data are expressed as means ± SEM. Key: (*) $P < 0.05$.

Protein content of each culture well was determined by the method of Markwell *et al.*, [12].

Data Analysis

Each experimental cell preparation is denoted by (N). For each cell preparation, hormone or cAMP production was determined from 3–6 separate dishes for a given condition and/or time point. The mean of such replicates was used as a single datum point for analysis. All data are expressed as means ± SEM. Due to wide variation among placental preparations, differences between ethanol-exposed and ethanol-naive (control) groups were assessed by nonparametric, paired analysis, namely the signed rank sum test. Differences were considered significant for $P < 0.05$. For the ethanol concentration-response, grouped data at each concentration were compared with controls by paired *t*-tests of log-transformed data.

RESULTS

Ethanol Concentration–Response for hCG Production

Trophoblasts were exposed to various concentrations of ethanol for 3 days, with daily medium change (Fig. 1). There was a concentration-dependent enhancement of 24-hr hCG production, up to and including the supraphysiologic concentration of 173 mM (800 mg/dL). For all

subsequent studies, we used a daily peak ethanol concentration of 65 mM.

Effect of 65 mM Ethanol on cAMP Production

In a separate set of experiments, intracellular cAMP levels were determined at 0, 2, 4, 8, 16, and 24 hr. At 2 hr, there was a significant increase in cAMP in ethanol-treated cells: control = 7.801 ± 1.079; ethanol = 9.617 ± 1.137 pmol/mg protein; $P < 0.02$, N = 7. At all other times, there was no difference in cAMP between ethanol-treated and control cells.

Effect of CTX or FSK on hCG and cAMP Production in Ethanol-Exposed Trophoblasts

To examine the effect of ethanol on hCG production when cells were treated with stimulators of cAMP production, trophoblasts were co-incubated with either CTX (100 ng/mL) or FSK (20 μM) during the final 20 hr of a 24-hr period. CTX stimulates cAMP production by ribosylating $G_s\alpha$ [13], which leads to a permanent activation of adenylyl cyclase. FSK stimulates cAMP production by activating the catalytic subunit of the adenylyl cyclase, partially by a G protein-dependent mechanism [14].

Ethanol treatment increased 24-hr hCG production over control values in the basal (unstimulated) state and when stimulated for 20 hr with CTX or FSK (Fig. 2a). In the basal state, the effect of ethanol was a 66% increase, whereas in the stimulated state, ethanol induced a 34 and 30% rise in hCG with CTX and FSK stimulation, respectively. The cAMP level at 24 hr was elevated after CTX or FSK treatment in both control and ethanol-treated cells. However, ethanol-treated cells exhibited a significantly greater cAMP response (50% higher with CTX and 91% with FSK) (Fig. 2b). Coupled with the findings of an early rise in cAMP in the basal state (see above), these data suggest that ethanol enhances cAMP production, which is reflected in the hCG response as well.

Effect of Ethanol Treatment on $G_s\alpha$ and $G_{i2}\alpha$ Concentration and Expression

The activity of adenylyl cyclase is regulated by G proteins, G_s or G_i. The increased cAMP production in ethanol-treated cells could have been due to increased expression of G_s and/or a decrease in G_i. The quantity of $G_s\alpha$ and $G_{i2}\alpha$ proteins from plasma membranes isolated from trophoblasts cultured with or without ethanol for 48 hr was determined by western blotting. As reported previously [15], the anti-$G_s\alpha$ antibody recognized both the 52-kDa and 45-kDa proteins that result from posttranscriptional modification of the gene product [16] in the trophoblast. The concentrations of the two forms of $G_s\alpha$ were not affected by ethanol treatment (Fig. 3a). In addition, the concentration of 40 kDa $G_{i2}\alpha$ was not altered by ethanol treatment. Similarly, the mRNA levels of $G_s\alpha$ or $G_{i2}\alpha$ were not affected by

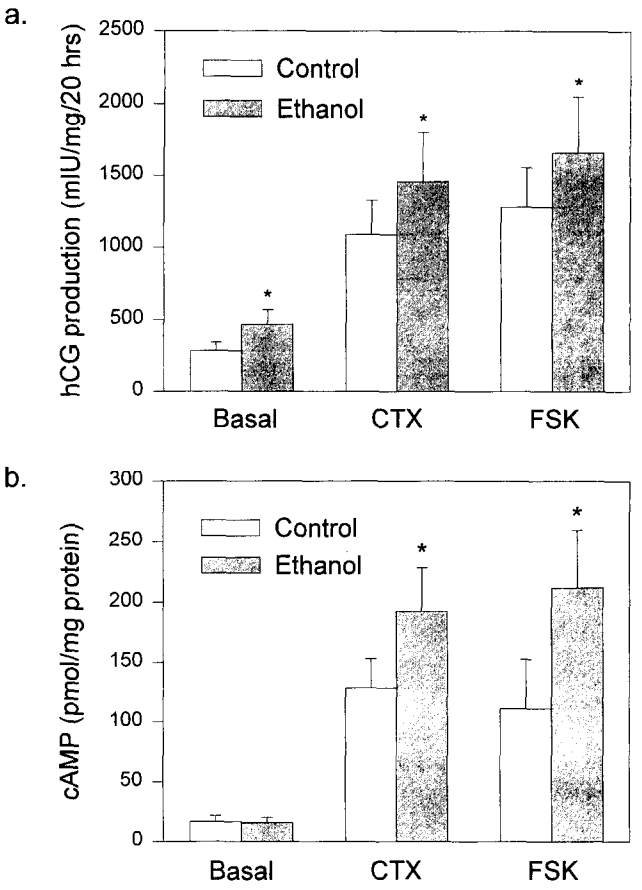

FIG. 2. Effect of CTX and FSK on hCG and cAMP levels in ethanol-exposed trophoblasts. Trophoblasts were exposed to ethanol (65 mM) for 3 days as described in Materials and Methods. During the final 20 hr, CTX (100 ng/mL) or FSK (20 μM) was included in the incubation medium. Following incubation, both the medium and cells were harvested and processed as described in the text for hCG assay (Fig. 2a, N = 6). Trophoblast-associated cAMP was determined as described in Materials and Methods (Fig. 2b, N = 5). Data are expressed as means ± SEM. Key: (*) $P < 0.02$ compared with paired ethanol-naive, untreated controls.

ethanol treatment in northern blot analyses (Fig. 3b). Therefore, in these non-transformed human cells, the changes in cAMP production induced by ethanol were not due to alterations in the expression of $G_s\alpha$ or $G_{i2}\alpha$.

Mn^{2+} Activated cAMP Production by Plasma Membranes Isolated from Ethanol-Treated Trophoblasts

In the absence of quantitative changes in the G protein, ethanol treatment may have altered adenylyl cyclase activity. Adenylyl cyclase activity was determined in plasma membranes prepared from trophoblasts cultured with or without ethanol for 48 hr. Cyclase was activated by a high concentration of Mn^{2+} (20 mM), which directly activates the catalytic subunit of adenylyl cyclase while uncoupling G protein-mediated activation. Ethanol-treated cells demonstrated greater cyclase activity (control = 45.7 ± 7.7; ethanol = 57.9 ± 8.5 pmol · mg^{-1} · min^{-1}; $P < 0.01$; N =

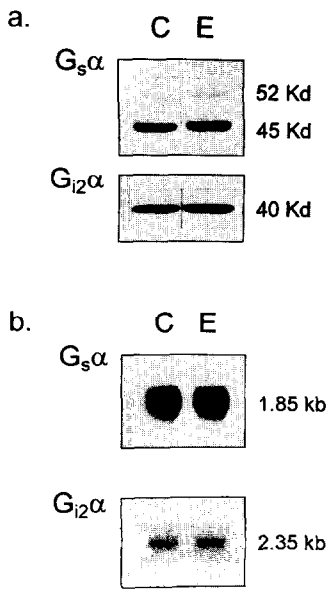

FIG. 3. Western and northern blot analyses of $G_s\alpha$ and $G_{i2}\alpha$ in ethanol-exposed trophoblasts. Cells were homogenized and processed as described in Materials and Methods to obtain the membrane fraction. Protein samples (50 μg) were solubilized in Laemmli sample buffer and subjected to SDS–PAGE, transferred to a nitrocellulose membrane, and incubated with antipeptide antibody for immunodetection of $G_s\alpha$ and $G_{i2}\alpha$ (Fig. 3a). Total RNA was extracted from ethanol-treated trophoblasts as described in Materials and Methods. RNA was resolved and transferred by northern blot to a Duralon-UV membrane (Stratagene). The filters were UV-cross-linked, prehybridized, and then hybridized overnight at 55° with radiolabeled cDNAs for human $G_s\alpha$ and rat $G_{i2}\alpha$ (Fig. 3b). No difference in loading was observed based upon the mRNA levels for glyceraldehydephosphate dehydrogenase (GAPDH) (data not shown).

10). cAMP production was higher in the membranes prepared from ethanol-treated cells by 27%. Similarly, when 20 mM Mn^{2+} and FSK (20 μM) were combined to enhance cAMP production, cAMP production was higher in membranes from ethanol-treated cells, by 21.5% (control = 232.5 ± 40.0; ethanol = 282.4 ± 32.3 pmol · mg^{-1} · min^{-1}; $P < 0.02$; N = 6). Therefore, ethanol treatment appears to increase adenylyl cyclase activity.

Effect of Rp-cAMP on hCG Production

To confirm that ethanol enhances hCG production via the cAMP pathway, cells were treated with Rp-cAMP. Rp-cAMP, which is an analog of cAMP, is a specific antagonist for cAMP activation of PKA [17]. In ethanol-naive cells, Rp-cAMP (3×10^{-5} M) reduced hCG production (Fig. 4a). In ethanol-exposed cells, Rp-cAMP abolished the ethanol-induced enhancement of hCG production (Fig. 4a).

Effect of 8-Br-cAMP on Ethanol-Enhanced hCG Production

Another mechanism by which ethanol treatment might enhance hCG production could be post-cAMP signaling,

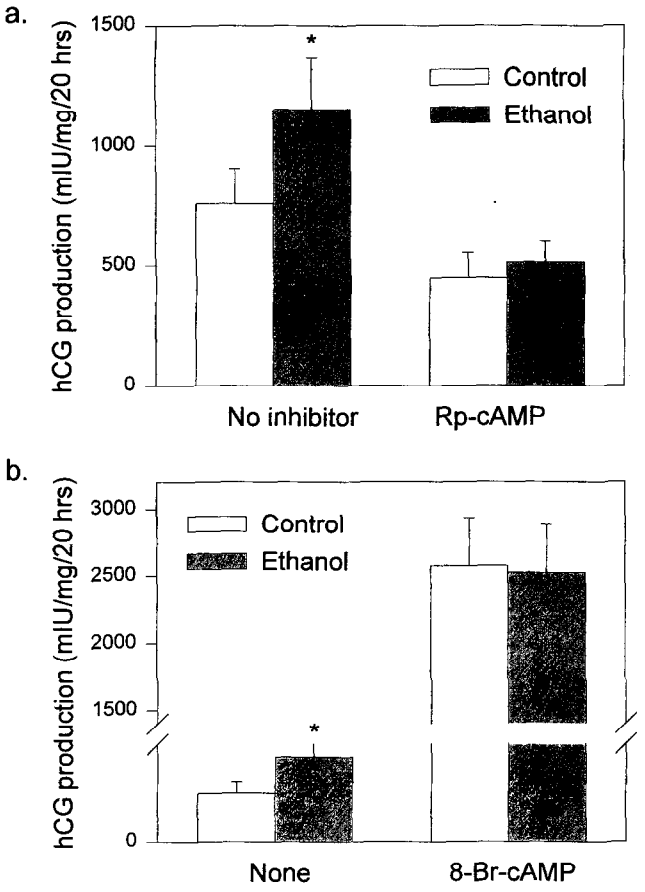

FIG. 4. Effect of (a) Rp-cAMP or (b) 8-Br-cAMP on ethanol-enhanced hCG production. Trophoblasts were exposed to ethanol for 72 hr. Control and ethanol-exposed cells were treated with Rp-cAMP (3×10^{-5} M) (N = 8) or 8-Br-cAMP (2 mM) for 20 hr (N = 6). Key: (*) $P < 0.05$ compared with ethanol-naive controls.

e.g. ethanol-induced increase in PKA activity. By saturating cells with a high concentration of a membrane-permeable cAMP, such as 8-Br-cAMP, PKA activity should become rate-limiting for subsequent signal transduction; if PKA activity is elevated, loading the ethanol-treated cells with a saturating concentration of cAMP should increase hCG production above control levels. When ethanol-naive control trophoblasts were incubated with a high concentration (2 mM) of 8-Br-cAMP during the final 20 hr of incubation, hCG production was increased by approximately 10-fold (Fig. 4b). When ethanol-treated cells were loaded with the same excess cAMP, the resulting hCG response was the same as in the controls. This suggests that ethanol treatment does not increase post-cAMP signaling, at least via PKA, and indicates that the effect of ethanol is related primarily to an enhancement of cAMP production.

DISCUSSION

Ethanol-induced perturbation of placental function could contribute to fetal injury in drinking women [reviewed in Ref. 3]. Previous studies in this laboratory have demon-

strated altered production of specific placental hormones [6] and cAMP [8] as a result of ethanol exposure. The current report extends these observations and demonstrates that ethanol-induced alterations in cAMP production are the basis for the increased hCG production by cultured trophoblasts. It provides an insight into one potential mechanism by which ethanol may cause placental dysfunction: enhancement of adenylyl cyclase activity. Moreover, we speculate that these disturbances in placental cell signaling could also occur in other fetal tissue.

The ethanol-induced increase in placental hCG production is dependent upon the concentration of ethanol, with peak enhancement at "physiologic" concentrations (43–87 mM; approximately 200–400 mg/dL). These concentrations are similar to those found in chronic alcoholics [18] and even some alcoholic women at the time of delivery [19]. This suggests that there may be similar *in vivo* perturbation of trophoblast function in women who regularly ingest alcohol.

Production of hCG is known to be cAMP-dependent in cultured placental cells [7]. Under basal (unstimulated) culture conditions, ethanol induced an early, transient rise in cAMP, which translated into increased hCG accumulation by 24 hr. When trophoblasts were exposed to stimulators of the cAMP-generating system (CTX or FSK), hCG production was increased in ethanol-treated cells, relative to ethanol-naive control cells. Conversely, inhibition of cAMP-mediated processes by Rp-cAMP or maximizing the cellular response by loading the cells with an excess of 8-Br-cAMP abolished the relative effect of ethanol on hCG accumulation. These observations suggest that the effect of ethanol is related to increased production of cAMP, rather than any post-cAMP pathway.

Accordingly, ethanol was found to boost the cAMP response in the stimulated state (either CTX or FSK). The enhanced response to CTX implies activation of adenylyl cyclase, perhaps by $G_s\alpha$. Yet, this occurred in the absence of any changes in the concentration of G proteins. This observation is in contrast to some reports in other cell types, where ethanol exposure affected cAMP production by altering expression of G_s [20, 21], or G_i [22, 23], or both [15]. However, functional activity of G_s may have been altered without changes in the protein concentration [14, 15]. There could have been post-translational modification of G proteins, i.e. ribosylation or phosphorylation [24–28], altering G protein function. Cholera toxin stimulates cAMP production by activation of G_s protein via ribosylation, whereas forskolin stimulates cAMP production by directly activating the catalytic subunit of adenylyl cyclase and/or through G protein-mediated mechanisms [14]. Our findings are consistent with ethanol-induced sensitization of G_s-dependent activation of adenylyl cyclase or a direct increase in adenylyl cyclase catalytic activity. Although ethanol might have somehow countered the inhibitory action of G_i on adenylyl cyclase, the ethanol effect appears to at least involve enhanced sensitivity to ribosylation of $G_s\alpha$ by cholera toxin. In addition, the activity of adenylyl cyclase, as determined by Mn^{2+}-activated activity, was higher in the membranes from ethanol-exposed trophoblasts. Taken together, the data suggest that either adenylyl cyclase activity *per se* or G protein-mediated activation of adenylyl cyclase (or both) may be altered by ethanol treatment.

Finally, another mechanism by which ethanol might alter the production of cAMP or hCG is by stimulating trophoblasts to secrete factors that secondarily affect signal transduction. To test this hypothesis, culture medium was collected from ethanol-treated cells. Ethanol was removed by bubbling with N_2. When ethanol-naive trophoblasts were exposed to this conditioned medium for 24 hr, isoproterenol-stimulated cAMP production was not affected, compared with control cells (data not shown). Therefore, ethanol-induced changes in ligand-stimulated cAMP production do not appear to be secondary to soluble factors released by ethanol-treated trophoblasts.

In summary, these experiments indicate that ethanol enhances cAMP production in normal human placental trophoblasts. The mechanism(s) responsible for increased cAMP and secondary hCG production, while appearing to at least involve enhanced adenylyl cyclase activity, remains to be elucidated.

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