PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **189**, 84–93 (1988)

Cyclic 3′,5′-Adenosine Monophosphate-Dependent Kinase and Phosphoproteins in Human Amnion[1] (42784)

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Abstract. 3′,5′-Cyclic adenosine monophosphate (cAMP) modulates prostaglandin production in human amnion membranes. The major effects of cAMP are presumably mediated through the phosphorylation of specific regulatory phosphoproteins following cAMP activation of cAMP-dependent protein kinase. Cyclic AMP-dependent protein kinase and phosphoproteins have not previously been characterized in human amnion. Total homogenates, cytosol, and membrane fractions from human amnion were examined for [^{3}H]cAMP binding activity and cAMP-dependent kinase activity. cAMP-dependent kinase activity was barely detectable in crude amnion fractions. Cytosol was therefore partially purified by DEAE column chromatography for further examination. Two peaks of coincident [^{3}H]cAMP binding and cAMP-dependent kinase activity were demonstrated at 70 and 140 m*M* NaCl, characteristic of the Type I and Type II cAMP-dependent protein kinase isozymes. [^{3}H]cAMP binding to the material from both peak fractions was saturable and reversible. Scatchard analysis of [^{3}H]cAMP binding to the peak fractions was linear for peak I and curvilinear for peak II. Assuming a one-site model, [^{3}H]cAMP binding to the Type I isozyme showed a $K_D = 4.17 \times 10^{-8}$ *M* and $B_{max} = 73$ pmole/mg protein; using a two-site model, [^{3}H]cAMP binding to the high-affinity site for the Type II isozyme had a $K_D = 3.94 \times 10^{-8}$ *M* and $B_{max} = 6.3$ pmole/mg protein. Other cyclic nucleotides competed for these [^{3}H]cAMP binding sites with a potency order of cAMP $\gg$ cGMP $>$ $(BU)_2$cAMP. cAMP caused a dose-dependent increase in cAMP-dependent kinase activity in the peak fractions; half-maximal activation was observed with 5.0×10^{-8} *M* cAMP. The ability of cAMP to increase phosphorylation of endogenous proteins in both crude amnion cytosol and cytosol from cultures of amnion epithelial cells was assessed using [^{32}P]ATP, SDS–polyacrylamide gel electrophoresis and autoradiography. cAMP stimulated ^{32}P incorporation into three proteins having M_r = 80,000, 54,000, and 43,000 ($P < .01$). Half-maximal ^{32}P incorporation into these proteins occurred at 1.0×10^{-7} *M* cAMP. cAMP-dependent kinase is present in human amnion; specific cAMP-enhanced phosphoproteins are also present. Hormones elevating cAMP levels in amnion may exert their effects by activating cAMP-dependent kinase and phosphorylating these phosphoproteins.

Human amnion has been postulated to be a target tissue for hormonal messages from the fetus concerning the timing of labor (1, 2). Numerous studies have shown that prostaglandin production in the amnion increases with gestation (3, 4), and with the labor process (5–8). Furthermore, proteins have been partially characterized in amniotic fluid and neonatal urine which are reportedly capable of increasing prostaglandin production in amnion tissue punches (9–12). Studies have also shown that the enzymes required for prostaglandin production in amnion rapidly increase specific activity near the end of gestation (13, 14). The hormone(s) which potentially controls these processes have not been determined.

A number of investigators have demonstrated that catecholamines and β-adrenergic agonists can increase prostaglandin production in amnion membranes and dispersed amnion cell preparations (15, 16). As catecholamine levels are very high in the fetus near parturition (17), and β-adrenergic agents are commonly used to inhibit the labor process (18), investigation of the amnion's potential to react to these agents through the β-adrenergic receptor/cAMP intracellular cascade system is of interest.

Catecholamines and β-adrenergic agents exert their effects by binding to cell membrane receptors and activating adenylate cyclase via an intermediary coupling protein

[1] This work was supported by PHS Grant HD20459.

0037-9727/88 $1.50

complex (G_s). Activated adenylate cyclase then produces cAMP which binds to and activates cAMP-dependent protein kinase. cAMP-dependent protein kinase phosphorylates specific cell proteins constituting the effect of the hormone in the cell (for review see (19, 20)).

β-adrenergic receptors have been characterized in human amnion (21) and have been shown to be coupled to catecholamine-sensitive adenylate cyclase (22). cAMP has been shown to have potential physiological effects in this tissue (15, 16). cAMP-dependent protein kinase and cAMP-dependent phosphoprotein substrates have not previously been characterized in amnion.

In this study cAMP-dependent kinase from human amnion was identified and partially characterized. Specific phosphoprotein substrates for this enzyme were also demonstrated in the amnion cytosol.

Materials and Methods. [γ-^{32}P]ATP (35 Ci/mmole) and [^{3}H]cAMP (36.1 Ci/mmole) were purchased from New England Nuclear Corp. (Boston, MA). cAMP-dependent protein kinase (bovine heart), cAMP-dependent protein kinase inhibitor (PKI), cAMP, cGMP, 3′,5′-dibutyryl cAMP [(Bu)$_2$ cAMP], and other reagents were purchased from Sigma Chemical Co. (St. Louis, MO). DEAE-cellulose was obtained from Whatman Inc. (Clifton, NJ). Cellulose acetate filters were from Millipore Corp. (Bedford, MA). Trypsin (223 μ/mg protein) was from Worthington Biomedical Corp. (Freehold, NJ). Fetal bovine serum was from Hazelton Research Products (Denver, PA). All other reagents were obtained from Fisher Scientific Co. (Cincinnati, OH).

Tissue preparation. Preparations made from amnion membranes. Placentas from uncomplicated pregnancies were obtained at delivery and processed immediately. Amnions were removed and washed in phosphate-buffered physiological saline. The tissue was then washed in ice-cold buffer containing 250 m*M* sucrose, 0.1 m*M* PMSF, 0.1 m*M* DTT, 1 m*M* EDTA, and 10 m*M* Tris–HCl (pH 7.2). Approximately 20 g of washed tissue was then homogenized with a Tekmar Tissuemizer (Tekmar Co., Cincinnati, OH) in 80 ml of the sucrose buffer (3 × 15 sec at high setting). The homogenate was poured through four layers of cheese cloth. Aliquots were then removed and used as the total homogenate preparation. The remaining material was centrifuged at 5000*g* for 10 min. The supernatant was centrifuged at 100,000*g* for 60 min. The supernatant of this centrifugation was used as the amnion cytosol preparation. The pellet of this final centrifugation was washed by resuspension in an equal amount of the sucrose buffer and finally resuspended in sucrose buffer to give a protein concentration of 2–3 mg/ml. Amnion fractions were assayed on the day of preparation or after freezing and storage at −70°C. Protein content of the preparation was determined by the method of Bradford *et al.* (23). Overnight dialysis was performed prior to some phosphorylation experiments to remove endogenous ATP and cAMP. There was no change in the results obtained.

Culture of amnion epithelial cells. Primary culture of amnion cells was done using a modification of the method of Okita *et al.* (24). Placentas were obtained from women undergoing repeat caesarian sections. All tissue manipulations were performed using aseptic technique and sterile (0.2 μm filtered) solutions. Amnion was stripped from chorion and transferred to a beaker containing 100 ml ice-cold phosphate-buffered saline, 30 μg/ml gentamicin (PBS). The tissue (30–50 g) was washed two more times with 100 ml portions of PBS until clear of blood and cut into strips. Tissue fragments were transferred to a 500-ml flask containing 50 ml 0.05% trypsin, 0.02% EDTA in calcium-free phosphate-buffered saline (TEPBS) and incubated 20 min at 37°C in a shaking waterbath. This preparation was filtered through a 2-mm stainless steel wire mesh. The filtrate was discarded and the tissue fragments transferred back to the flask. After addition of 150 ml TEPBS the fragments were incubated for 90 min at 37°C as before. The suspension was then combined with 500 ml ice-cold PBS, shaken vigorously for 60 sec and filtered consecutively through 2- and 0.7-mm meshes. The filtrate was centrifuged at 100*g* for 8 min at 4°C. Pelleted cells were resuspended at 1–5 × 10^5 cells/ml in minimum essential medium containing Earle's salts, supplemented with 10% fetal bovine serum, 1 m*M* sodium pyruvate, 2.2 g/liter

sodium bicarbonate, 10 m*M* Hepes (pH 7.1), 50 mg/liter gentamicin sulfate, 2.5 mg/liter amphotericin B (EMEM+), and plated in 150-mm diam tissue culture dishes (3–9 $\times 10^6$ cells/plate). Dishes were incubated at 37°C in an atmosphere of 95% air/5% CO_2/100% relative humidity. Medium was changed initially at 24 hr and at 48-hr intervals thereafter. Epithelial cell cultures were obtained by this procedure which showed no fibroblast contamination over several passes (1–2 months). The morphology of the cells under phase-contrast microscopy did not change with passage through at least three passes (as far as followed). To obtain cytosol, cells from the first or second pass were trypsinized, scraped with a rubber policeman, and thereafter centrifuged and resuspended in the above homogenization mixture. Cytosol was then prepared as for the intact membranes.

[^{3}H]cAMP binding assay. cAMP binding activity was determined by a method similar to that of Gilman (25) as previously reported (26). Triplicate samples containing approximately 90 μg protein were incubated at 4°C for 1.5 hr in 20 m*M* potassium phosphate buffer (pH 7.0) with varying concentrations of [^{3}H]cAMP (0.5–100 n*M*). Under these conditions, equilibrium binding of [^{3}H]cAMP to the receptor was achieved. The 200-μl assay volume was then precipitated with 0.5 ml saturated $(NH_4)_2SO_4$ and filtered through HAWP 0.45 Millipore filters (Millipore Corp., Bedford, MA). The filters were washed with 30 ml (3×10 cm^3) 20 m*M* phosphate buffer (pH 7.0), dried, and counted by standard scintillation technique. Nonspecific binding was assessed in identical assays containing 1 m*M* unlabeled cAMP. Nonspecific binding was less than 1% of the total binding near the K_D (10 n*M*).

Displacement studies were performed at 2×10^{-8} *M*[^{3}H]cAMP with varying concentrations of cAMP, cGMP, $(Bu)_2$cAMP, or other nucleotides added to the incubation mixture. The percentage displacement of [^{3}H]cAMP binding by an agent was calculated from binding in the absence of the agent (100%), and binding in the presence of 1 m*M* cAMP (0%). The K_D values were determined from the ED_{50} by the method of Cheng and Prusoff (27).

Scatchard analysis. Binding data analysis was done with an IBM AT computer using a nonlinear model fitting program (Ligand-PC) graciously provided by Peter J. Munson of the Laboratory of Theoretical and Physical Biology, NICHD (28).

Protein kinase assay. Protein kinase activity was measured by a modification of the method of Gill (29) as previously reported (26). The reaction mixture (total volume 0.2 ml) consisted of the protein sample (50–150 μg), 10 m*M* $MgCl_2$, 10 m*M* NaF, 0.5 m*M* EGTA, and 20 m*M* Tris–HCl (pH 7.2), histone II A (1 mg/ml) and 0.05 m*M* [γ-^{32}P]ATP (5–20 cpm/pmole). cAMP, cGMP, and protein kinase inhibitor (0.2 mg/ml) were added to some assays. Prior to its use in any assays, PKI was purified with a DEAE-cellulose column by the method of Kanter and Brunton (30) to remove contaminating kinases. Incubations were carried out for 5 min at 30°C and the reaction terminated by the addition of 50 μl of 2.5% bovine serum albumin (BSA) in 0.02% deoxycholate followed by 2.5 ml of 2.5% trichloroacetic acid at 4°C. The mixture was then filtered through glass fiber filters (GF/F) which had been presoaked in 10% trichloroacetic acid and 2.5% sodium pyrophosphate. The filters were washed with an additional 7.5 ml of 2.5% trichloroacetic acid, dried, and counted by standard scintillation technique. Under these conditions, protein phosphorylation was linear with respect to incubation time and to amnion protein concentration.

DEAE-cellulose chromatography. DEAE-cellulose chromatography (DE-52, Whatman, Inc.) was performed on a 2×30-cm column equilibrated with 10 m*M* potassium phosphate (pH 7.0). Samples were placed on the column, which was then washed with 1000 ml phosphate buffer. The column was developed with a continuous gradient (0–500 m*M*; 250 ml each) of NaCl at a buffer flow rate of 0.5–1 ml/min. Seven-milliliter fractions were collected and assayed for conductivity, protein concentration, [^{3}H]cAMP binding, and protein kinase activity. Fractions were stored frozen and used within 2 months without appreciable change in activity.

The relative amounts of the regulatory subunits (Types I and II) of cAMP-depen-

dent protein kinase were determined by plotting specific [^{3}H]cAMP binding per milliliter elutate and comparing the areas of the two resultant peaks.

Gel electrophoresis and autoradiography. Phosphorylation substrates were identified by incubation of amnion fractions with [γ-^{32}P]ATP followed by gel electrophoresis and autoradiography as previously described (31). In these experiments the reaction mixture (total volume 0.2 ml) consisted of the protein sample (20–30 μg), 5 μM [γ-^{32}P]ATP (5–20 cpm/pmole), 20 mM Tris–HCl (pH 7.2), 5 mM $MgCl_2$, 1 mM EGTA, and 10 mM NaF, plus added cAMP. cAMP-dependent protein kinase inhibitor (0.2 mg/ml), purified as described in the protein kinase assay above, was also added to specified assays. Assays were initiated by addition of [γ-^{32}P]ATP and incubated for 5 min at 30°C in a water bath. The reaction was terminated by the addition of 200 μl of 12% trichloroacetic acid at 4°C. The protein was then precipitated and the pellet washed twice with 350 μl H_2O). The pellet was then dissolved in a solution (75 μl) containing 2% SDS, 10% glycerol, 5% β-mercaptoethanol, and 0.001% bromphenol blue, and boiled for 5 min. Samples were then loaded on an SDS–polyacrylamide gel (stacking %T 4.4%, %C 2.6%; resolving %T 9.7%, %C 2.6%) for electrophoresis according to the method of Laemmli (32). The molecular weight standards used included myosin (205,000), β-galactosidase (116,000), phosphorylase b (97,400), BSA (66,000), ovalbumin (45,000), and carbonic anhydrase (29,000). The gels were fixed overnight in 50% methanol and 7.5% acetic acid, stained with coomassie brilliant blue, and destained. Gels were dryed on Whatman grade 3 filter paper, autoradiographed on Kodak XR film, and developed after varying periods (24 h to 2 weeks) to demonstrate the phosphorylated proteins. The autoradiographs were then scanned at 550 nm by a Beckman Du-8 spectrophotometer with slab gel scanner attachment.

RESULTS. *Localization.* cAMP-dependent protein kinase activity was determined in amnion total homogenates, microsomal membranes, and cytosol. As illustrated in Table I, cAMP-dependent kinase activity was detected only in amnion cytosol. The basal level of histone phosphorylation was higher in microsomal membranes than in the cytosol but cAMP only increased histone phosphorylation in the cytosol. Because of the relatively low activity of cAMP-dependent kinase detected in all crude fractions of human amnion relative to that reported in placenta (26) and other tissues (31), the remainder of the studies of cAMP-dependent protein kinase were performed on DEAE-cellulose purified material.

TABLE I. cAMP-DEPENDENT PROTEIN KINASE ACTIVITY IN AMNION FRACTIONS

	Basal (pmole PO_4/mg protein/min)	Activity ratio
Crude homogenate	0.92 ± 0.38	0.57 ± 0.10
Microsomal membrane	3.95 ± 1.29	0.99 ± 0.16
Cytosol	0.45 ± 0.18	0.33 ± 0.14

Note. Basal activity in the absence of cAMP and activity ratio [−cAMP/+cAMP] are shown. Values represent the mean ± SEM for fractions from six amnions. Phosphorylation of histone protein by amnion fractions was carried out for 5 min as described under Methods.

DEAE-cellulose chromatography of amnion cytosol. Crude amnion cytosol was fractionated by DEAE-cellulose chromatography. cAMP-dependent kinase activity and [^{3}H]cAMP binding activity eluted in two peaks (Fig. 1) at approximately 70 and 140 mM NaCl corresponding to the Type I and the Type II cAMP-dependent protein kinase seen in many other tissues. Approximately 45% of the total cAMP-dependent kinase activity was present as the Type I and 55% as the Type II isozyme. Ninety percent of the cAMP stimulatable kinase activity from crude cytosol was recovered in the two peaks. In order to further characterize the cAMP-dependent protein kinase isozymes, DEAE column fractions containing each peak of binding and kinase activity were pooled (hereafter referred to as peak I and peak II fractions).

Characterization of partially purified amnion cAMP-dependent kinase. Protein kinase activity increased with increasing cAMP concentration from a basal level of 340 pmole/ml/min $^{32}PO_4$ incorporated at 10^{-10}

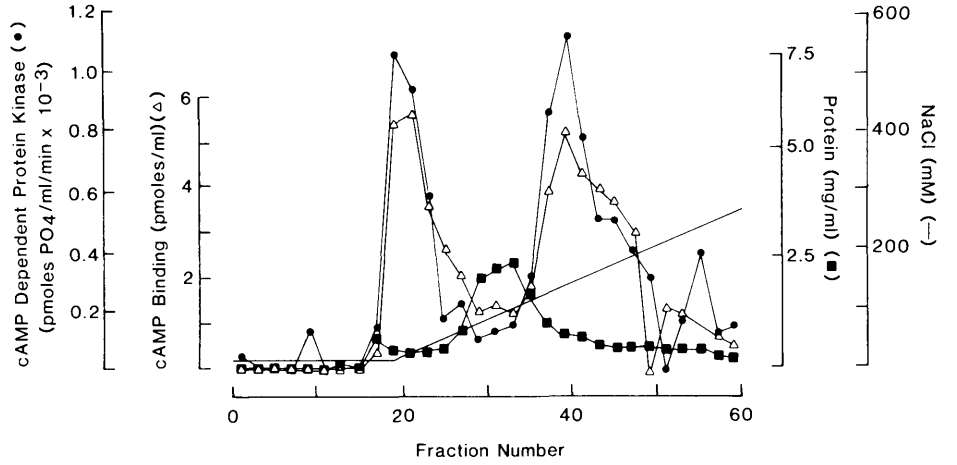

FIG. 1. DEAE-cellulose chromotography of amnion cytosol. Amnion cytosol (400 ml of 1.5 mg protein/ml concentration) was loaded onto a 1.5 × 20-cm column equilibrated with 10 m*M* potassium phosphate. The column was washed and developed with a linear NaCl gradient, as described under Materials and Methods. Protein kinase activity in the presence of 10^{-6} *M* cAMP, [^{3}H]cAMP binding, NaCl concentration, and protein are shown on the vertical axis. The fraction number is indicated on the horizontal axis. The designations I and II refer to protein kinase isozymes I (fractions 16–27) and II (fractions 34–47).

M cAMP to 406 pmole/ml/min at 10^{-5} *M* cAMP in pooled fractions from peak I and from 225 pmole/ml/min to 363 pmole/ml/min in pooled fractions from peak II (Fig. 2). Binding of [^{3}H]cAMP to pooled peak fractions was saturable in the range of tissue cAMP levels (10). The best fitting one- or two-site model was used for analyses of [^{3}H]cAMP binding to each peak fraction (Fig. 3). A one-site model provided the best fit for the binding data for peak I. [^{3}H]cAMP binding to peak I fractions showed a K_D of 4.17×10^{-8} *M* and B_{max} of 63 pmole/mg protein. A two-site model best fit the binding data for peak II. With a two-site model a K_D of 3.94×10^{-8} *M* and B_{max} of 6.3 pmole/mg protein was obtained for the high-affinity site, and a K_D of 4.99×10^{-7} *M* and B_{max} of 26.3 pmole/mg protein was obtained for the low-affinity site. The specificity of [^{3}H]-cAMP binding was determined by measuring the displacement of [^{3}H]cAMP by varying concentrations of unlabeled nucleotides (Fig. 4). As expected, cAMP was considerably more potent than cGMP or $(Bu)_2$cAMP in displacing [^{3}H]cAMP. The K_D's of cAMP, cGMP, and $(Bu)_2$cAMP were respectively 2.97×10^{-8}, 1.16×10^{-5}, 3.49×10^{-5} *M* for peak I and 5.68×10^{-9}, 1.14×10^{-6}, and 1.17×10^{-5} *M* for peak II. Adenosine AMP, ADP, ATP, and GTP (all at 10^{-4} *M*) had no effect on [^{3}H]cAMP binding.

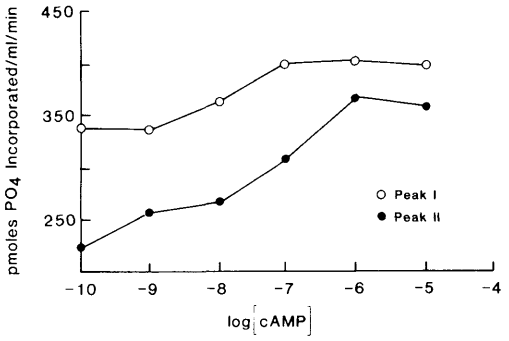

FIG. 2. cAMP dose response of amnion cytosol phosphorylation of histone protein. Amnion (40 μg protein) peak I (open circle) and peak II (filled circle) were incubated for 5 min at 30°C in the manner described under Materials and Methods for protein kinase assay, except that the cAMP concentration was varied from 10^{-10} to 10^{-5} *M*. The logarithm of [cAMP] is indicated on the horizontal axis. Kinase activity, in picomoles of $^{32}PO_4$ incorporated into histone protein per milliliter amnion peak fraction, is indicated on the vertical axis. The experiment shown is representative of three experiments done from peak fractions from three different fractioning experiments (Fig. 1).

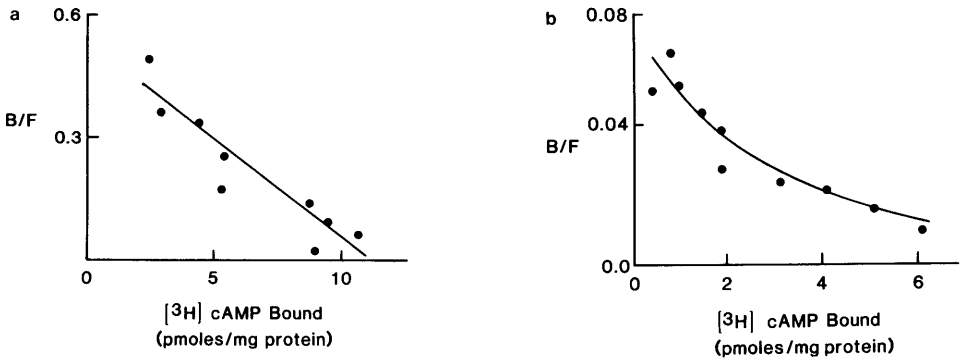

FIG. 3. Scatchard analysis of [^{3}H]cAMP binding to amnion peak I (a) and peak II (b). The B_{max} and K_D were calculated by nonlinear analysis of the binding data as described under Methods. The results are representative of three experiments (n = 3).

The ability of cAMP to increase the phosphorylation of endogenous phosphoproteins was examined in amnion cytosol, microsomal membranes, and total amnion homogenates. In each preparation 20–25 phosphorylated proteins were resolved on the gel, however, cAMP enhancement of phosphorylation was seen only in the cytosol. cAMP enhanced the phosphorylation of three proteins in amnion cytosol (M_r = 80,000, 54,000, and 43,000) (Fig. 5a). Densitometer scans of autoradiographs of amnion cytosol phosphorylated in the presence versus absence of cAMP (10^{-6} M) demonstrated significant increases (paired t test, $P < 0.01$; n = 8) in the phosphorylation of each of these proteins (Table II). In addition, cAMP increased the phosphorylation of phosphoproteins with M_r = 221,000 and 27,400 in several preparations. Phosphorylation of all of these proteins was inhibited by cAMP-dependent protein kinase inhibitor (PKI). The effect of PKI was not totally specific for the cAMP-dependent phosphoproteins, however. PKI also inhibited the phosphorylation of other proteins in the cytosol whose phosphorylation was not enhanced by cAMP (data not shown). Dose-dependent phosphorylation of the cAMP-dependent phosphorylation is shown in Fig. 6. Half-maximal phosphorylation occurs at 1.0×10^{-7} M cAMP.

The detected activity of cAMP-dependent kinase in amnion is relatively low. In order to assure that cAMP-dependent kinase and its phosphoprotein substrates are present in

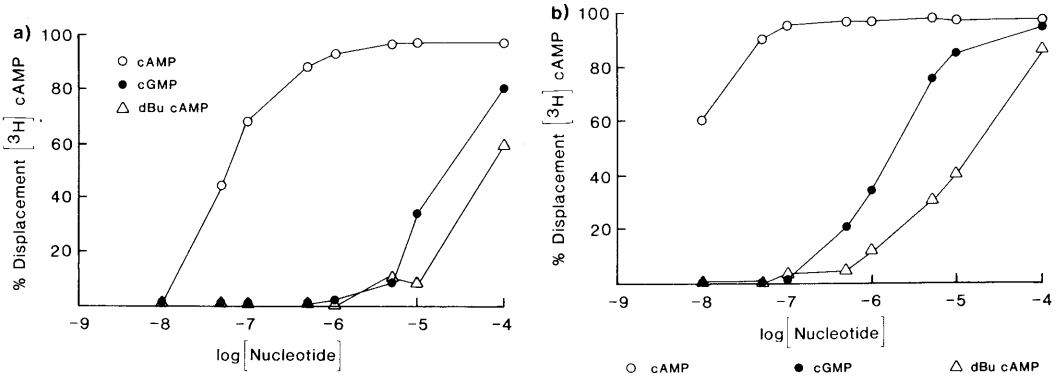

FIG. 4. Inhibition of [^{3}H]cAMP binding to amnion peak I (a) and peak II (b). [^{3}H]cAMP (3×10^{-8} M) plus cold nucleotides at varying concentrations added simultaneously were incubated for 90 min at 4°C, as described under Materials and Methods. The results are representative of three experiments (n = 3).

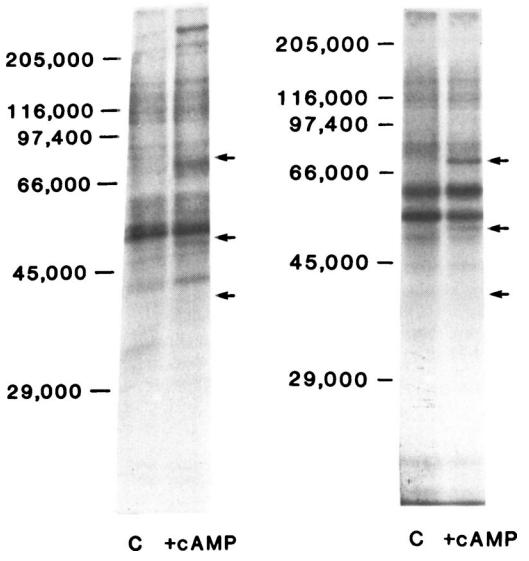

FIG. 5. Autoradiograph of phosphorylated proteins in amnion cytosol (a) and cytosol from amnion epithelial cells (b). Cytosol was incubated as described under Methods with or without cAMP (10^{-6} M) as indicated. The arrows indicate differentially phosphorylated proteins. The positions of marker proteins are indicated to the left. The results shown are representative of gels from amnion cytosol of eight placentas (n = 8) and three cultures (n = 3).

amnion epithelial cells and not merely in contaminating fibroblasts or cells floating in amniotic fluid, cytosol produced from human amnion epithelial cells in culture was also tested. Although these cultured cells may not behave identically to the same cells *in situ*, cAMP increased the phosphorylation of phosphoproteins of molecular mass similar to those seen in the whole membrane cytosol preparation (Fig. 5b).

Discussion. cAMP-dependent kinase activity and cAMP-dependent phosphorylation of specific proteins were identified and partially characterized in human amnion. The specific activity of cAMP-dependent kinase in human amnion was noted to be lower than in many previously studied tissues so that partial purification of the enzyme was required for even preliminary study. When purified on DEAE-cellulose, human amnion cAMP kinase exhibited two peaks of cAMP binding and cAMP responsive protein kinase activity with characteristics of the Type I and the Type II isozyme described in other tissues. Studies of the enzyme's ability to phosphorylate endogenous proteins revealed that three amnion cytosol proteins (M_r = 80,000, 54,000, and 43,000) showed increased phosphorylation in the presence of physiological levels of cAMP (10^{-6} M). cAMP also increased phosphorylation of proteins of similar molecular mass in cytosol from cultures of amnion epithelial cells. This phosphorylation was inhibited by cAMP-dependent protein kinase inhibitor. The three amnion proteins therefore respond similarly in activator and inhibitor studies to phosphoproteins in other tissues which have been shown to be phosphorylated specifically by cAMP-dependent kinase (35–37).

Other amnion proteins may also be phosphorylated by cAMP-dependent protein kinase. Several amnion proteins, while not showing increased phosphorylation with cAMP addition, showed decreased phosphorylation with cAMP-dependent protein kinase inhibitor. These proteins may be maximally phosphorylated by basal levels of endogenous cAMP-dependent kinase in placenta. However, they may also be dephosphorylated by some nonspecific effect of the inhibitor. Still other cAMP-dependent proteins may lie below the range of detectability of the gels used in these experiments (approximately 15,000 mol wt) and may therefore have remained undetected. Finally, dephosphorylation of some proteins may occur quickly enough for their initial phosphorylation to be undetectable.

TABLE II. INCORPORATION OF ^{32}P INTO PHOSPHOPROTEINS BY cAMP

cAMP-dependent phosphoprotein (M_r)	cAMP-induced density increase (percent of control)
80,000	460 ± 80
54,000	228 ± 34
43,000	245 ± 70

Note. Densitometry readings (arbitrary) for the cAMP-dependent phosphoproteins from eight amnions cytosol preparations are given. M ± SEM. Phosphorylation, SDS–Polyacrylamide gel electrophoresis and densitometry of amnion cytosol were performed as described under Methods.

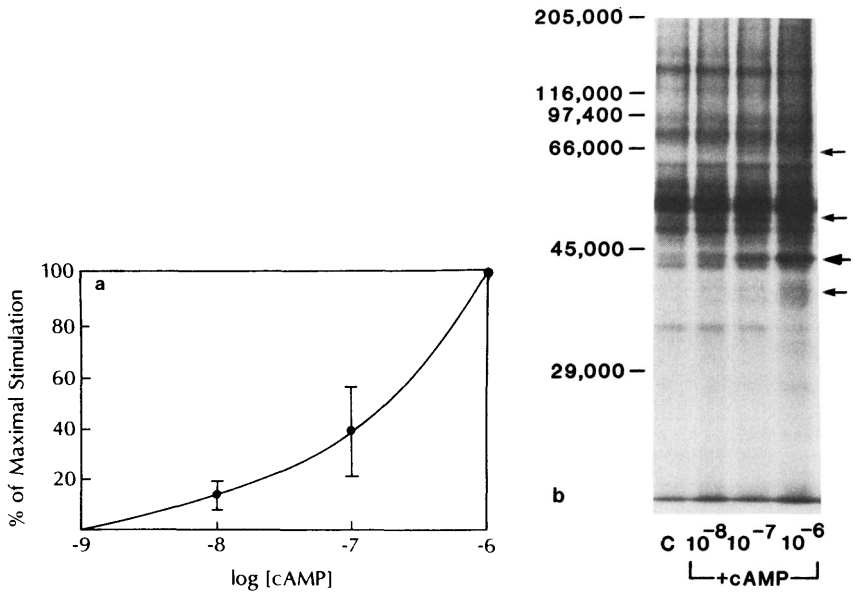

FIG. 6. Dose response of phosphorylation of the 43,000 M_r protein. The degree of phosphorylation of the 43,000 M_r protein as determined by densitometer scanning of autoradiographs (vertical axis) is shown versus concentration of cAMP (a), and a representative autoradiograph is shown in (b). The arrows indicate differentially phosphorylated proteins. The positions of marker proteins are indicated to the left. The results are representative of three experiments (n = 3).

Hormones and agents which increase intracellular cAMP levels in amnion and other tissues have been reported to increase prostaglandin production in the amnion. In numerous studies using β-adrenergic agonists, cholera toxin, forskolin, and cAMP analogs the magnitude of this increase has been disappointing (less than twofold) in the context of the hypothesis that prostaglandin production by amnion plays a major role in labor (16). In recent experiments, using the above activators in primary cultures of amnion cells we have also seen no better than a twofold increase in PGE_2 production. In contrast, agents stimulating the C-kinase system increase PGE_2 output more than 50-fold (38). The mechanism of action for cAMP-dependent processes in amnion is presumably mediated through phosphorylation of target proteins by cAMP-dependent protein kinase. In human amnion this enzyme required purification before its binding or kinase activity was equal to that seen in crude fractions of other tissues. The specific activity of this enzyme in the human amnion may therefore not support a major role for cAMP-dependent protein kinase and its activators (e.g., β-agonists) in prostaglandin production leading to labor.

Cyclic AMP-dependent protein kinase and three cAMP-dependent phosphoproteins have been preliminarily characterized in amnion homogenates. None of the phosphoproteins have been identified, nor has a connection been established between any of them and cAMP or catecholamine-dependent processes previously described in amnion explant and cell culture studies. However, the entire cascade for catecholamine action through the β-adrenergic system, including β-2 receptors (21), catecholamine sensitive adenylate cyclase (16, 22), cAMP-dependent protein kinase, and cAMP-dependent phosphoproteins has now been established in human amnion. This cascade may, therefore, be a mechanism by which the previously reported effects of catecholamines and β-agonists upon amnion metabolism may occur.

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Received March 21, 1988. P.S.E.B.M. 1988, Vol. 189.
Accepted June 16, 1988.