Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 10619–10623, November 1995
Medical Sciences

Serine phosphorylation of human P450c17 increases 17,20-lyase activity: Implications for adrenarche and the polycystic ovary syndrome

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Communicated by Melvin M. Grumbach, University of California, San Francisco, CA, July 21, 1995 (received for review May 26, 1995)

ABSTRACT **Microsomal cytochrome P450c17 catalyzes both steroid 17α-hydroxylase activity and scission of the C17—C20 steroid bond (17,20-lyase) on the same active site. Adrenal 17α-hydroxylase activity is needed to produce cortisol throughout life, but 17,20-lyase activity appears to be controlled independently in a complex, age-dependent pattern. We show that human P450c17 is phosphorylated on serine and threonine residues by a cAMP-dependent protein kinase. Phosphorylation of P450c17 increases 17,20-lyase activity, while dephosphorylation virtually eliminates this activity. Hormonally regulated serine phosphorylation of human P450c17 suggests a possible mechanism for human adrenarche and may be a unifying etiologic link between the hyperandrogenism and insulin resistance that characterize the polycystic ovary syndrome.**

The synthesis of sex steroids from pregnenolone or progesterone first requires the 17α-hydroxylation of these steroids to 17α-hydroxypregnenolone and 17α-hydroxyprogesterone, respectively. The C17—C20 carbon bond of either of these 21-carbon steroids is then cleaved to yield the 19-carbon androgens dehydroepiandrosterone (DHEA) and androstenedione, respectively, which are then converted to testosterone and estradiol. Both the 17α-hydroxylation of pregnenolone and progesterone and the subsequent C17—C20 bond cleavage (17,20-lyase activity) of 17α-hydroxypregnenolone and 17α-hydroxyprogesterone are catalyzed by a single enzyme, cytochrome P450c17. Only a single enzyme catalyzing 17α-hydroxylase activity and 17,20-lyase activity can be isolated from steroidogenic tissues (1–4), and transfection of vectors expressing the cDNAs for either bovine (5) or human (6) P450c17 confers both 17α-hydroxylase and 17,20-lyase activity to the transfected cells. There is only one human gene for P450c17 (7) located on chromosome 10q24-q25 (8), and mutations of this gene can destroy all 17α-hydroxylase and 17,20-lyase activity (6, 9–12). Thus it is clear that there is only one enzyme catalyzing both of these activities.

In the testis, all of the precursor steroids are converted to sex steroids—i.e., the effective ratio of lyase activity to hydroxylase activity is unity. However, in the human adrenal cortex, this ratio is under closely regulated control during development. The adrenals of children between 1 and 8 years of age make cortisol (a C_{21} steroid) but virtually no C_{19} steroids. Between 7 and 9 years, the adrenals begin to produce DHEA associated with increased adrenal 17α-hydroxylase and 17,20-lyase activity (13) and without an associated change in the secretion of cortisol or adrenocorticotropic hormone (14, 15). DHEA secretion increases steadily, reaching maximal levels at 25–35 years, and afterwards wanes slowly, returning to childhood levels at 70–80 years (16). This onset of adrenal androgen synthesis, an event termed adrenarche, is independent of the gonads or pituitary gonadotropins (17) and only occurs in humans and other higher primates (14). While it has been suggested that adrenal androgens are under the control of a specific pituitary tropic hormone (15, 17), no such hormone has been found (18–20). Other efforts have focused on possible intraadrenal events such as adrenal mass, blood flow, and intra-adrenal steroid concentrations that might affect adrenal androgen production (21). An especially intriguing approach was suggested by the observation that increasing the molar ratio of isolated, purified electron donors, such as P450 oxidoreductase (OR) or cytochrome b_5, to porcine P450c17 would increase the ratio of lyase to hydroxylase activity (22). Experiments with transfected COS-1 cells confirm that coexpression of vectors encoding human OR and human P450c17 results in a substantial increase in 17,20-lyase activity (23). However, it seems unlikely that adrenarche could result from a large increase in the expression of an electron donor, as the activity of adrenal cytochrome P450c21 (steroid 21-hydroxylase), which uses the same electron donors, is unchanged during adrenarche.

Studies of the contribution of various residues in P450c17 suggest that specific amino acids are uniquely required for 17,20-lyase activity: the mutation R346A in rat P450c17 (24) or the corresponding mutant R347A in human P450c17 (25) destroys virtually all 17,20-lyase activity while having minimal effects on 17α-hydroxylase activity. Other subtle mutations can also affect this ratio (23–26). As the amino acid sequence of P450c17 cannot change with adrenarche, we have thus considered whether posttranslational modification of P450c17 could alter the ratio of hydroxylase to lyase activity.

We now report that serine phosphorylation of human P450c17 appears to be required for 17,20-lyase activity. We hypothesize that this phosphorylation is developmentally regulated in the adrenal cortex in response to as yet unidentified stimuli, thus suggesting a mechanism for the onset of adrenarche. Furthermore, the association of serine phosphorylation with the induction of androgen synthesis suggests a unifying hypothesis for the two cardinal features of some forms of the polycystic ovary syndrome (i.e., hyperandrogenism and insulin resistance), as serine phosphorylation of the insulin receptor can cause insulin resistance (27, 28).

MATERIALS AND METHODS

Cell Culture and Immunoprecipitations. COS-1 African green monkey kidney cells were grown in monolayer in 10-cm dishes in 10 ml of Dulbecco's modified Eagle's medium/Ham's 21 (DME-H21) containing 4.5 g of glucose per liter and 10% fetal bovine serum and were transfected as described (6). NCI-H295 human adrenocortical carcinoma cells were grown as described (29, 30) and were transfected by calcium phos-

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Abbreviations: DHEA, dehydroepiandrosterone; OR, P450 oxidoreductase; PKA, protein kinase A; IGF-I, insulin-like growth factor I.

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phate precipitates. Kin 8 cells (31), a mutant of mouse adrenocortical Y1 cells lacking the regulatory subunit of protein kinase A (PKA), were generously provided by Bernard P. Schimmer and were grown as described (32).

Cells were metabolically labeled either with [^{32}P]orthophosphate ($^{32}P_i$) (1 mCi/ml; 1 Ci = 37 GBq; free of carrier and HCl; Amersham) for 2–4 h in phosphate-free DME-H21 or with [^{35}S]methionine (0.1 mCi/ml, >37 TBq/mmol; Amersham) for 1–4 h in methionine-free DME-H21 containing 10% dialyzed fetal calf serum. Labeled cells were lysed in 10 mM Tris·HCl, pH 7.5/20 mM sodium pyrophosphate/20 mM nitrophenyl phosphate/1% deoxycholate/1% Triton X-100/0.5% bovine serum albumin/1 mM phenylmethylsulfonyl fluoride/200 μM sodium vanadate/aprotinin, leupeptin, and pepstatin A each at 20 μg/ml/cystatin at 10 μg/ml. The lysates containing [^{35}S]methionine and $^{32}P_i$ contained 0.1% and 1.2% SDS, respectively. Lysates were clarified by centrifugation for 30 min at 140,000 × *g*. The supernatant was precleared with protein A-Sepharose CL-4B beads (Pharmacia) before adding antiserum to human P450c17 (23) at a dilution of 1:300. The immune complexes were harvested with protein A-Sepharose CL-4B beads, washed extensively, and analyzed by electrophoresis on SDS/10% polyacrylamide gels. Western immunoblotting of one- and two-dimensional gels was done as described (23, 33).

Plasmids, Transfections, and Enzyme Assays. The human P450c17 cDNA (34) was subcloned into the expression vector pMT2 (35). The expression vector for the catalytic subunit of cAMP-dependent PKA (RSV-Catβ) (36) was generously provided by Richard A. Maurer. Plasmids (2 μg) purified by CsCl banding were transfected overnight into COS-1 cells by calcium phosphate precipitates as described (37). After changing the medium, cells were incubated 48 h, washed twice with PBS, washed twice with DME-H21 lacking phosphate or methionine, and then were used for metabolic labeling as above or for enzymatic hormonal conversion assays. Enzymatic assays used [^{3}H]pregnenolone (925 GBq/mmol; DuPont/NEN) or 17α-[^{3}H]hydroxypregnenolone (618 GBq/mmol; Amersham) in DME-H21 containing 10% fetal calf serum. Steroids were extracted and analyzed by TLC exactly as described (6, 23).

Phosphoamino Acid Analysis. ^{32}P-labeled human P450c17 was isolated by immunoprecipitation with rabbit anti-human P450c17 and protein A-Sepharose CL-4B as described above, then displayed by electrophoresis on an SDS/10% polyacrylamide gel, and electroblotted to nitrocellulose membranes. The P450c17 band was cut out, digested with trypsin, then hydrolyzed with 5.7 M HCl, and analyzed by thin-layer electrophoresis in two dimensions at pH 1.9 and pH 3.5. Autoradiograms were compared to the ninhydrin-stained plates, all as described (38, 39).

Microsomal Assays. Human fetal adrenal microsomes were prepared by homogenizing 300 mg of tissue in 2 ml of 0.25 M sucrose/5 mM EDTA, pH 7.4, clearing debris at 9000 × *g* for 20 min, and centrifuging at 105,000 × *g* for 1 h. The crude pellet was washed in 0.1 M K_xPO_4 buffer, pH 7.4/0.1 mM EDTA; the microsomes were harvested at 105,000 × *g* for 1 h, resuspended in 3 ml of 0.1 M K_xPO_4 buffer/0.1 mM EDTA/20% (vol/vol) glycerol, and stored at −20°C. The protein content was 2.6 mg/ml, and the P450 content measured by CO-difference spectra using a millimolar extinction of $\Delta\varepsilon_{450-490}$ = 91 $mM^{-1}\cdot cm^{-1}$ (40) was 0.99 nmol/mg of protein. P450c17 activity was measured by incubating 12 μg of microsomal protein and 1 nmol of [^{3}H]pregnenolone in 0.2 ml of 100 mM K_xPO_4 (pH 7.4), 1 mM $MgCl_2$, 0.4 mM $NADP^+$, 5 mM glucose 6-phosphate, and 0.2 unit of glucose-6-phosphate dehydrogenase at 37°C for 30 min. Steroids were extracted with 0.8 ml of ethyl acetate in 2,2,4-trimethylpentane (1:1, vol/vol), concentrated by evaporation, and analyzed by TLC in benzene/acetone (8:1, vol/vol). OR activity was assayed by measuring the rate of reduction of 50 μM cytochrome *c* by 11.2 μg of microsomal protein in 100 μl of 0.3 M K_xPO_4, pH 7.4/50 μM NADPH, assessed at 550 nm using a millimolar extinction of 21 $mM^{-1}\cdot cm^{-1}$ (41).

RESULTS

Human P450c17 Is a Phosphoprotein. To determine if there might be posttranslational modification of P450c17 that would influence its charge (e.g., phosphorylation) more than its molecular mass, we performed two-dimensional gel electrophoresis of proteins extracted from human fetal adrenals and identified P450c17 isoforms by Western blotting. The majority of the immunodetectable P450c17 was found in a single spot, but a small amount of P450c17 was also seen with a somewhat more basic isoelectric point (data not shown). Because some hepatic (42, 43) and steroidogenic (44, 45) P450 enzymes may be phosphorylated, we used human adrenal NCI-H295 cells to determine if the charge variants of P450c17 were due to phosphorylation. NCI-H295 cells make C_{19} steroids and 17-hydroxylated C_{21} steroids (29) and express abundant P450c17 mRNA (30) and immunodetectable protein. NCI-H295 cells were labeled with either $^{32}P_i$ or [^{35}S]methionine for 2.5 h and then treated for another 2 h with or without 200 μM 8Br-cAMP, and the P450c17 protein was immunoharvested with rabbit anti-human P450c17 and staphylococcal protein A-Sepharose and analyzed by SDS/polyacrylamide gel electrophoresis. The 2-h treatment with 8Br-cAMP increased incorporation of [^{35}S]methionine, an index of total P450c17 protein, by about one-third, but incorporation of $^{32}P_i$, an index of phosphorylation, increased 4-fold (Fig. 1). Thus it appears that P450c17 is phosphorylated in response to cAMP in NCI-H295 cells.

P450c17 Is Phosphorylated by a cAMP-Dependent Protein Kinase. To elucidate the mechanisms of P450c17 phosphorylation further, we used nonsteroidogenic COS-1 cells transfected with vectors expressing human P450c17. Our pECE-based expression vector for P450c17 expresses useful levels of P450c17 activity (6, 10, 11, 23, 25) but yields modest levels of immunodetectable P450c17 protein. Therefore we built a new P450c17 expression vector in pMT2 (35), which expressed substantially more P450c17 and which confirmed that P450c17 could be phosphorylated in COS-1 cells (Fig. 2). When COS-1 cells transfected with this vector were stimulated for 2 h with 200 μM 8Br-cAMP, there was no change in immunoprecipitable [^{35}S]P450c17, but ^{32}P-labeling of P450c17 increased 3-fold (Fig. 3, lanes 1–7). Similarly, when this P450c17 vector was cotransfected with a vector expressing the catalytic subunit of PKA (RSV-Catβ) (36), the incorporation of $^{32}P_i$ increased about 2-fold (Fig. 3, lanes 8 and 9). By contrast, incubation for 2 h with phorbol 12-myristate 13-acetate at 20 ng/ml did not alter ^{32}P incorporation (lanes 10 and 11). Thus the phosphorylation of P450c17 in COS-1 cells appears to be catalyzed by a cAMP-dependent protein kinase. To determine if PKA phosphorylates P450c17, we performed the same experiment in Kin 8 cells, a derivative of mouse adrenocortical Y1 cells that

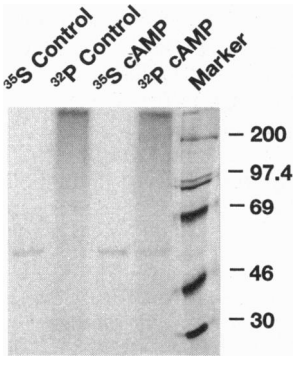

FIG. 1. Labeling of P450c17 in human adrenal NCI-H295 cells. Cells (2 × 10^6) were labeled for 2.5 h with [^{35}S]methionine or $^{32}P_i$ and then for another 2 h without (Control) or with (cAMP) 200 μM 8Br-cAMP. The cells were lysed and the immunoharvested P450c17 was displayed on an SDS/10% polyacrylamide gel. Size markers are in kDa. The data are representative of three experiments.

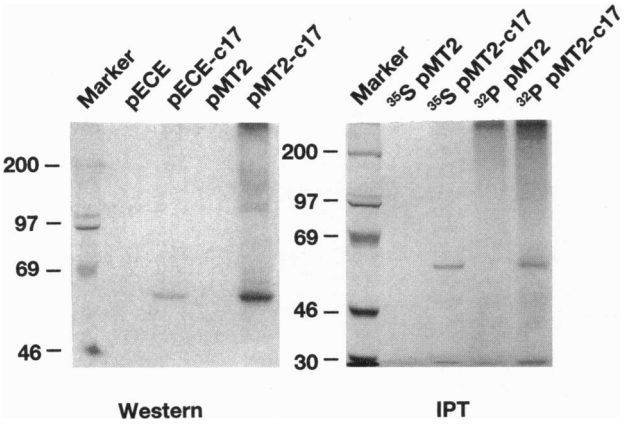

FIG. 2. Expression of P450c17 in transfected COS-1 African green monkey kidney cells. (*Left*) Western blot of proteins from 2×10^6 cells transfected for 48 h with pECE vector, pECE expressing P450c17 (pECE-c17), pMT2 vector, or pMT2 expressing P450c17 (pMT2-c17). (*Right*) Immunoprecipitation (IPT) of P450c17 from 2×10^6 cells transfected with pMT2 or pMT2-c17 for 48 h and incubated with [^{35}S]methionine or $^{32}P_i$ for 4 h and 200 μM 8Br-cAMP for 1 h. Size markers are in kDa. A single Western blot and two immunoprecipitation experiments were done.

lack the regulatory subunit of PKA (32). After transfection with the P450c17 expression vector, incubation of Kin 8 cells with $^{32}P_i$ yielded no detectable ^{32}P-labeled P450c17, and cotransfection with RSV-Catβ yielded minimally detectable ^{32}P-labeled P450c17 (lanes 12 and 13). Thus P450c17 appears to be phosphorylated in a cAMP-dependent fashion, but PKA appears to play a relatively minor role in this phosphorylation.

P450c17 Is Phosphorylated at Serine (and Threonine) Residues. The apparent role of PKA in P450c17 phosphorylation would suggest that P450c17 might be phosphorylated at serine and possibly threonine residues. To determine the chemical identity of the phosphorylated residues, we performed two-dimensional phosphoamino acid analysis (39). Direct HCl treatment of the P450c17 phosphoprotein isolated from an SDS gel did not yield complete hydrolysis; however, when the protein was first digested with trypsin, followed by HCl, complete hydrolysis was achieved. As shown in Fig. 4, about 70–80% of the ^{32}P was associated with serine residues, and the remainder was associated with threonine residues,

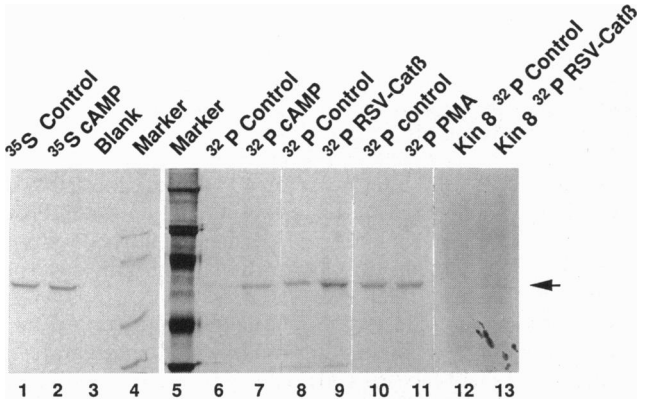

FIG. 3. Induction of P450c17 phosphorylation. COS-1 cells or Kin 8 cells (lanes 12 and 13) were transfected with pMT2-c17 for 48 h; in lanes 9 and 13, cells were cotransfected with RSV-Catβ. Transfected cells were labeled for 2 h with [^{35}S]methionine or $^{32}P_i$ and then incubated another 2 h without (Control) or with 200 μM 8Br-cAMP (lanes 2 and 7) or phorbol 12-myristate 13-acetate at 20 ng/ml (lane 11). Size markers are in kDa; the P450c17 band is indicated by the arrow. Lanes 1–9 were done four times, lanes 10–13 were done once.

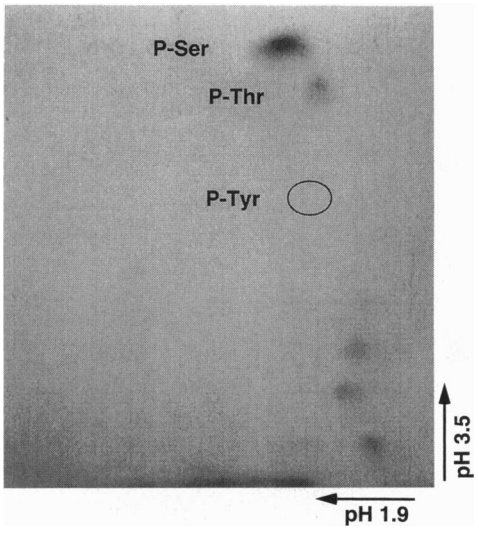

FIG. 4. Two-dimensional phosphoamino acid analysis. Phosphorylated P450c17 was prepared by immunoprecipitation from COS-1 cells transfected with pMT2-c17 and treated with $^{32}P_i$ and 200 μM 8Br-cAMP for 4 h. After tryptic digestion and HCl hydrolysis, electrophoresis along the ordinate at pH 1.9 was for 45 min at 1.6 kV and along the abscissa at pH 3.5 for 35 min at 1.3 kV. After autoradiography, the various amino acid spots were identified by ninhydrin staining.

whereas there was no detectable radioactivity associated with tyrosine residues.

Phosphorylation of P450c17 Increases 17,20-Lyase Activity. Our previous data show that cotransfection of a vector expressing OR, the protein that normally donates electrons to P450c17, into COS-1 cells with a P450c17 expression vector will increase the 17,20-lyase activity of the expressed P450c17 (23). Incubation of transfected COS-1 cells with $^{32}P_i$ showed that cotransfection with the OR expression vector did not increase or decrease the phosphorylation of P450c17 (data not shown). To assess the influences of OR and of PKA, cells were incubated with [^{14}C]pregnenolone, and the production of 17α-hydroxypregnenolone and DHEA were assessed by TLC and scintillation counting of the appropriate spots. Cotransfection with either RSV-Catβ or OR increased the 17,20-lyase activity of P450c17, but the combination of OR and RSV-Catβ did not increase lyase activity further (Fig. 5). Thus phosphorylation of P450c17 appears to increase the efficiency of electron transfer, possibly by increasing the affinity of P450c17 for OR.

Dephosphorylation of P450c17 Reduces 17,20-Lyase Activity. The induction of 17,20-lyase activity in cells treated with cAMP was often difficult to assess because untreated cells retained some 17,20-lyase activity and some phosphorylation of P450c17. Therefore, we also assessed the effect of dephosphorylation of P450c17 *in vitro*. Microsomal preparations of transfected cells did not contain enough total P450 to permit measurement by CO-difference spectra (data not shown), but microsomes from human fetal adrenal tissue yielded about 1 nmol of P450 protein per milligram of total protein. When adrenal microsomes were incubated with [^{3}H]pregnenolone or 17α-[^{3}H]hydroxypregnenolone, they exhibited both 17α-hydroxylase and 17,20-lyase activity. However when microsomal proteins were dephosphorylated with alkaline phosphatase, the microsomes lost 17,20-lyase activity, but not 17α-hydroxylase activity, in a time-dependent fashion (Fig. 6). As P450c17 activity requires electrons donated by microsomal OR, we determined whether alkaline phosphatase affected this enzyme. Even twice the amount of alkaline phosphatase used above had no effect on OR activity (Fig. 6*B*). The persistence of 17α-hydroxylase and OR activities after treat-

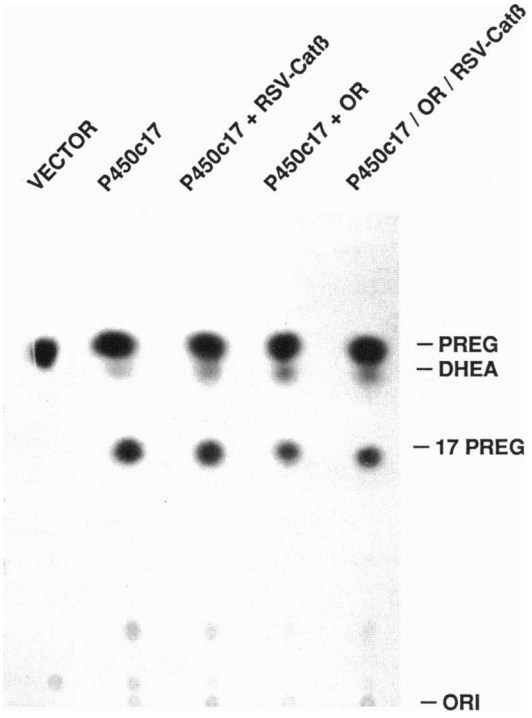

FIG. 5. Induction of 17,20-lyase activity. COS-1 cells were transfected with pMT2-c17, pMT2-c17 plus RSV-Catβ, pMT2-c17 plus pECE-OR, or all three expression vectors and were then incubated with [^{14}C]pregnenolone for 3 h. The resulting steroids were assayed by TLC. Equal counts were applied to each lane. The data are representative of four experiments. PREG, pregnenolone; 17 PREG, 17α-hydroxypregnenolone; ORI, origin.

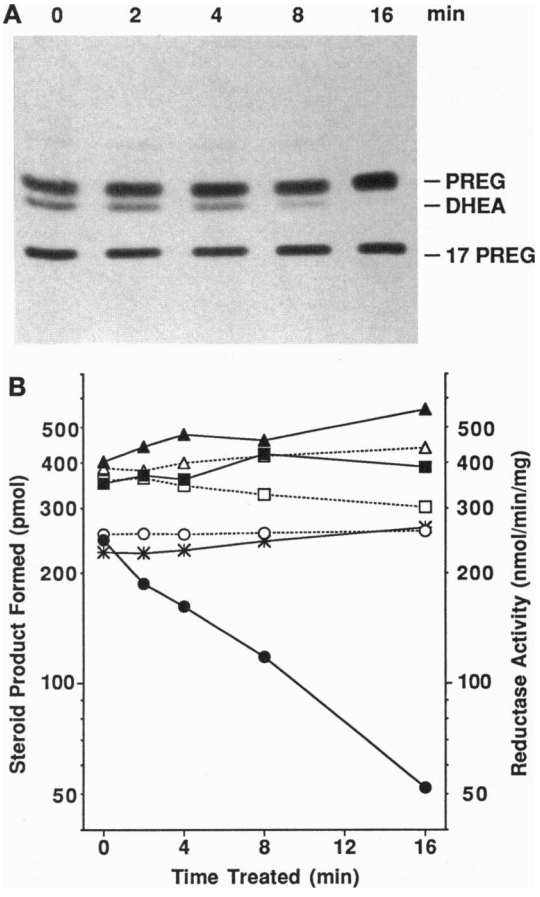

FIG. 6. Enzymatic activities of human fetal adrenal microsomes. One hundred thirty micrograms of microsomal protein was combined in 100 μl of 50 μM Tris·HCl, pH 8.0/1 mM $MgCl_2$ and preincubated 3 min at 37°C; then 0.88 unit of alkaline phosphatase was added, and a 19-μl aliquot was taken at 0, 2, 4, 8, and 16 min and transferred to an ice-cold tube containing 2 μl of 50 mM EDTA to stop the phosphatase reaction. Controls used H_2O instead of alkaline phosphatase. The activities of P450c17 were then assayed by incubating each batch of microsomes with [^{3}H]pregnenolone for 30 min. (*A*) TLC analysis of steroids produced by microsomes treated with alkaline phosphatase. PREG, pregnenolone; 17 PREG, 17α-hydroxypregnenolone. (*B*) Products formed (in pmol) calculated from the radioactivity of excised bands. Open symbols, controls; closed symbols, treated with alkaline phosphatase; △ and ▲, pregnenolone; □ and ■, 17α-hydroxypregnenolone; ○ and ●, DHEA. Data are the means of duplicate determinations. Also shown in *B* is the insensitivity of OR to alkaline phosphatase (*—*; right-hand scale). The experiment was performed as in *A* except that 1.76 units of alkaline phosphatase was used. Data are the means of duplicate determinations from one of two independent experiments.

ment with alkaline phosphatase shows that the loss of 17,20-lyase activity was not due to proteolysis [as suggested (45)]. Similarly the persistence of 17α-hydroxylase activity with both pregnenolone and progesterone substrates, an activity that requires NADPH, shows that the alkaline phosphatase-induced loss of 17,20-lyase activity was not due to dephosphorylation of this cofactor. Alkaline phosphatase treatment also did not alter the substrate-induced difference spectrum of total microsomal P450 incubated with either pregnenolone or 17α-hydroxypregnenolone (Fig. 7). Thus alkaline phosphatase treatment did not degrade P450c17, and dephosphorylation of P450c17 diminished 17,20-lyase activity without altering the substrate binding.

DISCUSSION

Adrenarche is a developmentally programmed increase in 17,20-lyase activity. To date, no mechanism for adrenarche has been proposed that accounts for a large increase in 17,20-lyase activity without altering 17α-hydroxylase activity. We now propose that serine/threonine phosphorylation of adrenal P450c17 by a cAMP-dependent kinase can account for the available observations concerning adrenarche and several related phenomena. Previous studies show that additional reducing equivalents are required for 17,20-lyase activity—e.g, from P450 oxidoreductase or cytochrome b_5 (22, 23, 46, 47). The phosphorylation of P450c17 obviously does not directly provide the increased reducing equivalents needed for 17,20-lyase activity but may increase the affinity of P450c17 for the electron donor. A related example appears to be the R346A mutation in rat P450c17 (R347A in human), which selectively destroys 17,20-lyase activity while retaining 17α-hydroxylase activity (24, 25). Computer graphic modeling predicts that this residue is not part of the catalytic substrate-binding pocket but instead will be exposed to solvent on the surface of the proximal face of the enzyme where it would participate in the initial charge recognition and binding between P450 reductase and the enzyme (25, 48). Similarly, phosphorylation of P450c17 may increase its electrostatic affinity for electron donors, thus favoring 17,20-lyase activity.

While the physiologic trigger to adrenarche and/or P450c17 phosphorylation remains unknown, we would speculate that insulin-like growth factor I (IGF-I) may be a good candidate. Serum levels of IGF-I rise and fall in a pattern that is contemporaneous with DHEA secretion. Both insulin and IGF-I transmit their signals by initiating tyrosine autophosphorylation of the insulin/IGF-I receptors, whereas the phosphorylation of serine and threonine residues diminishes signal transduction (27, 28). Patients with the polycystic ovary syndrome have hyperinsulinism and a postreceptor form of insulin resistance without a change in insulin binding, apparently in

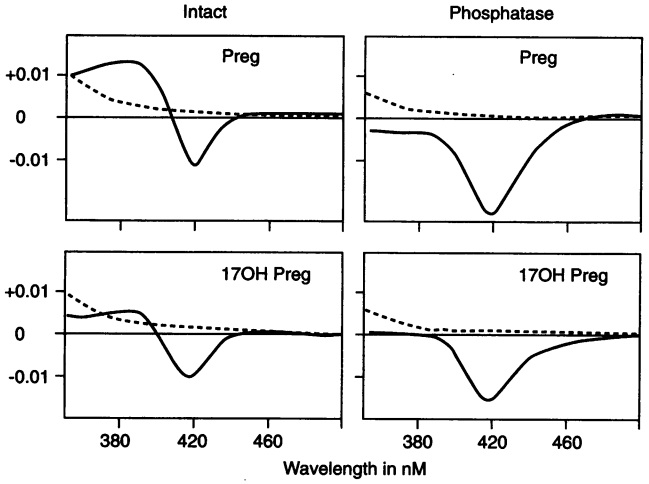

FIG. 7. Substrate-induced difference spectra. Microsomes (300 μg of protein) were used intact or were incubated with 5.28 units of alkaline phosphatase for 8 min, treated with EDTA, washed twice with 100 mM potassium phosphate buffer followed by a 60-min centrifugation at 105,000 × *g*, and resuspended in 100 mM potassium phosphate/20% glycerol at 1.48 mg of protein per ml. Substrate-induced spectra were recorded 3 min after addition of 10 μM pregnenolone (Preg) or 17α-hydroxypregnenolone (17OH Preg) to untreated (intact) or phosphatase-treated microsomes containing 0.5 μM P450 protein. Data are from a single experiment.

association with increased serine phosphorylation of the insulin receptor (49). We hypothesize that a common pathway results in excessive serine phosphorylation of the insulin receptor in a wide variety of tissues and of adrenal and ovarian P450c17 in patients with the polycystic ovary syndrome, thus increasing adrenal and ovarian 17,20-lyase activity, causing hyperandrogenism, and decreasing insulin sensitivity by a common mechanism. The nature of the responsible kinase(s) is unknown. The insulin receptor can undergo serine phosphorylation in response to several isozymes of protein kinase C (28), by cAMP-dependent kinases (50), by a casein kinase (51), and possibly by the insulin receptor itself (52). Our results suggest that a cAMP-dependent mechanism, but not a phorbol 12-myristate 13-acetate-sensitive protein kinase C mechanism, will phosphorylate P450c17 in adrenal cells, but other kinases may function in ovarian theca cells. Thus, while normal adrenal phosphorylation of P450c17 might be triggered at adrenarche, abnormal activation of serine phosphorylation, perhaps in response to dystonic secretion of luteinizing hormone might simultaneously increase (*i*) the serine phosphorylation of ovarian P450c17, causing ovarian hyperandrogenism, and (*ii*) the serine phosphorylation of insulin receptors, causing insulin resistance, thus providing a common pathway for the two principal features of some forms of the polycystic ovary syndrome.

We thank Dr. John E. Shively for productive discussions, Dr. Richard A. Maurer (University of Oregon) for the RSV-Catβ vector, and Dr. Bernard P. Schimmer (University of Toronto) for the Y1 and Kin 8 cells. This work was supported by a fellowship from the Cheng Scholars Program (L.Z.), National Institutes of Health physician/scientist award K11,DK02123 (H.R.), a grant from the College of Pharmacy Nihon University and a research fellowship from the Uehara Memorial Foundation (S.O.), and National Institutes of Health Grants DK37922 and DK42154 and March of Dimes Grant 6-396 (W.L.M.).

1. Nakajin, S. & Hall, P. F. (1981) *J. Biol. Chem.* **256,** 3871–3876.
2. Nakajin, S. & Hall, P. F. (1981) *J. Biol. Chem.* **256,** 6134–6139.
3. Nakajin, S., Shively, J. E., Yuan, P. & Hall, P. F. (1981) *Biochemistry* **20,** 4037–4042.
4. Nakajin, S., Shinoda, M., Haniu, M., Shively, J. E. & Hall, P. F. (1984) *J. Biol. Chem.* **259,** 3971–3976.
5. Zuber, M. X., Simpson, E. R. & Waterman, M. R. (1986) *Science* **234,** 1258–1261.
6. Lin, D., Harikrishna, J. A., Moore, C. C. D., Jones, K. L. & Miller, W. L. (1991) *J. Biol. Chem.* **266,** 15992–15998.
7. Picado-Leonard, J. & Miller, W. L. (1987) *DNA* **6,** 439–448.
8. Sparkes, R. S., Klisak, I. & Miller, W. L. (1991) *DNA Cell Biol.* **10,** 359–365.
9. Yanase, T., Simpson, E. R. & Waterman, M. R. (1991) *Endocrinol. Rev.* **12,** 91–108.
10. Fardella, C. E., Zhang, L. H., Mahachoklertwattana, P., Lin, D. & Miller, W. L. (1993) *J. Clin. Endocrinol. Metab.* **77,** 489–493.
11. Fardella, C. E., Hum, D. W., Hornoki, J. & Miller, W. L. (1994) *J. Clin. Endocrinol. Metab.* **79,** 160–164.
12. Monno, S., Ogawa, H., Date, T., Fujioka, M., Miller, W. L. & Kobayashi, M. (1993) *J. Biol. Chem.* **268,** 25811–25817.
13. Schiebinger, R. J., Albertson, B. D., Cassorla, F. G., Bowyer, D. W., Geelhoed, G. W., Cutler, G. B. & Loriaux, D. L. (1981) *J. Clin. Invest.* **67,** 1177–1182.
14. Cutler, G. B., Glenn, M., Bush, M., Hodgen, G. D., Graham, C. E. & Loriaux, D. L. (1978) *Endocrinology* **103,** 2112–2118.
15. Parker, L. N. & Odell, W. D. (1980) *Endocr. Rev.* **1,** 397–410.
16. Orentreich, N., Brind, J. L., Rizer, R. L. & Vogelman, J. H. (1984) *J. Clin. Endocrinol. Metab.* **59,** 551–555.
17. Sklar, C. A., Kaplan, S. L. & Grumbach, M. M. (1980) *J. Clin. Endocrinol. Metab.* **51,** 548–556.
18. Mellon, S. H., Shively, J. E. & Miller, W. L. (1991) *J. Clin. Endocrinol. Metab.* **72,** 19–22.
19. Penhoat, A., Sanchez, P., Jaillard, C., Langlois, D., Begeot, M. & Saez, J. M. (1991) *J. Clin. Endocrinol. Metab.* **72,** 23–26.
20. Robinson, P., Bateman, A., Mulay, S., Spencer, S. J., Jaffe, R. B., Solomon, S. & Bennett, H. P. J. (1991) *Endocrinology* **129,** 859–867.
21. Couch, R. M., Muller, J. & Winter, J. S. D. (1986) *J. Clin. Endocrinol. Metab.* **63,** 613–618.
22. Yanagibashi, K. & Hall, P. F. (1986) *J. Biol. Chem.* **261,** 8429–8433.
23. Lin, D., Black, S. M., Nagahama, Y. & Miller, W. L. (1993) *Endocrinology* **132,** 2498–2506.
24. Kitamura, M., Buczko, E. & Dufau, M. L. (1991) *Mol. Endocrinol.* **5,** 1373–1380.
25. Lin, D., Zhang, L., Chiao, E. & Miller, W. L. (1994) *Mol. Endocrinol.* **8,** 392–402.
26. Koh, Y., Buczko, E. & Dufau, M. L. (1993) *J. Biol. Chem.* **268,** 18267–18271.
27. Takayama, S., White, M. F. & Kahn, C. R. (1988) *J. Biol. Chem.* **263,** 3440–3447.
28. Chin, J. E., Dickens, M., Tavare, J. M. & Roth, R. A. (1993) *J. Biol. Chem.* **268,** 6338–6347.
29. Gazdar, A. F., Oie, H. K., Shackleton, C. H., Chen, T. R., Triche, T. J., Myers, C. E., Chrousos, G. P., Brennan, M. F., Stein, C. A. & LaRocca, R. V. (1990) *Cancer Res.* **50,** 5488–5496.
30. Staels, B., Hum, D. W. & Miller, W. L. (1993) *Mol. Endocrinol.* **7,** 423–433.
31. Rae, P. A., Butmann, N. S., Tsao, J. & Schimmer, B. P. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1896–1900.
32. Schimmer, B. P. (1985) *Methods Enzymol.* **109,** 350–356.
33. Black, S. M., Szklarz, G. D., Harikrishna, J. A., Lin, D., Wolf, C. R. & Miller, W. L. (1993) *Endocrinology* **132,** 539–545.
34. Chung, B., Picado-Leonard, J., Haniu, M., Bienkowski, M., Hall, P. F., Shivley, J. E. & Miller, W. L. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 407–411.
35. Kaufman, R. J., Davies, M. V., Pathak, V. K. & Hershey, J. W. B. (1989) *Mol. Cell. Biol.* **9,** 946–958.
36. Maurer, R. A. (1989) *J. Biol. Chem.* **264,** 6870–6873.
37. Moore, C. C. D., Brentano, S. T. & Miller, W. L. (1990) *Mol. Cell. Biol.* **10,** 6013–6023.
38. Boyle, W. J., van der Geer, P. & Hunter, T. (1991) *Methods Enzymol.* **201,** 110–149.
39. Luo, K., Hurley, T. R. & Sefton, B. M. (1991) *Methods Enzymol.* **201,** 149–152.
40. Omura, T. & Sato, R. (1964) *J. Biol. Chem.* **239,** 2379.
41. Van Gelder, B. F. & Slater, E. C. (1962) *Biochim. Biophys. Acta* **58,** 593–595.
42. Oesch-Bartlomowicz, B. & Oesch, F. (1990) *Arch. Toxicol.* **64,** 257–261.
43. Johansson, I., Eliasson, E. & Ingelman-Sundberg, M. (1991) *Biochem. Biophys. Res. Commun.* **174,** 37–42.
44. Vilgrain, I., Defaye, G. & Chambaz, E. M. (1984) *Biochem. Biophys. Res. Commun.* **125,** 554–561.
45. Bellino, F. L. & Holben, L. (1989) *Biochem. Biophys. Res. Commun.* **162,** 498–504.
46. Onoda, M. & Hall, P. F. (1982) *Biochem. Biophys. Res. Commun.* **108,** 454–460.
47. Aoyama, T., Nagata, K., Yamazoe, Y., Kato, R., Matsunaga, E., Gelboin, H. V. & Gonzalez, F. J. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 5425–5429.
48. Hasemann, C. A., Kurumbail, R. G., Boddupalli, S. S., Peterson, J. A. & Deisenhofer, J. (1995) *Structure* **3,** 41–62.
49. Dunaif, A. (1995) *Am. J. Med.* **98,** Suppl. 1A, 33S–39S.
50. Stadtmauer, L. & Rosen, O. M. (1986) *J. Biol. Chem.* **261,** 3402–3407.
51. Rapuano, M. & Rosen, O. M. (1991) *J. Biol. Chem.* **266,** 12902–12907.
52. Baltensperger, K., Lewis, R. E., Woon, C. W., Vissavajjhala, P., Ross, A. H. & Czech, M. P. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 7885–7889.