Protein kinase C signal transduction pathway in ACTH-induced growth effect of rat adrenocortical cells in primary culture

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Abstract

ACTH exerts a biphasic effect on the growth of fetal rat adrenocortical cells in primary culture when bromodeoxyuridine (BrdU) incorporation is used as an indicator of proliferation. The immediate inhibitory effect during the first 24 h of ACTH stimulation is not dependent on cyclic AMP (cAMP). Protein kinase C (PKC) inhibitors H-7 and staurosporine blocked this inhibitory effect of ACTH, whereas 12-0-tetradecanoyl phorbol-13-acetate (TPA; a PKC activator) mimicked the ACTH-induced antimitogenic effect. The stimulatory growth effect of ACTH appears after 72 h of treatment. A similar mitogenic effect is also achieved with cAMP derivative 8-bromo cAMP (8-Br cAMP). However, both ACTH- and 8-Br cAMP-induced proliferations could be reduced with H-7.

ACTH-induced corticosterone secretion was inhibited 50% with H-7 after 24 h, but 8-Br cAMP-induced secretion was unaffected. However, if the treatments were continued for 72 h, H-7 no longer reduced the steroid secretions. Reduction (50–75%) of cholesterol side-chain cleavage enzyme (P450sc) mRNA expression was also noted with H-7 in ACTH-treated cultures after 6 and 24 h. In contrast, TPA doubled the corticosterone secretion induced by 8-Br cAMP, but did not further increase the ACTH-induced secretion after 24 h. TPA alone, however, was not able to induce steroid secretion or P450sc mRNA expression. The morphological differentiation of fetal rat adrenocortical cells with ACTH or 8-Br cAMP from zona glomerulosa-like cells into zona fasciculata-like cells was not disturbed by H-7 nor was it induced by TPA alone.

These results therefore suggest that PKC- and cAMP-dependent signal transductions are involved in the ACTH-induced biphasic growth effect of fetal rat adrenocortical cells. PKC plays a role in the inhibitory growth effect, and both PKC and cAMP are involved in the stimulatory growth phase of ACTH. Both PKC and cAMP are also involved in the steroid secretion of zona glomerulosa-type cells, but differentiation into zona fasciculata-type cells and their steroid production is transduced through cAMP.


Introduction

Cyclic AMP (cAMP) is known to be the principal mediator of the effects of adrenocorticotropic hormone (ACTH) in the adrenal cortex. ACTH-like effects on the differentiation and steroidogenesis of adrenocortical cells are producible with cAMP derivatives (Garren et al. 1971, Nussdorfer et al. 1973, Armato et al. 1975, Hornsby & Gill 1977, Saez et al. 1981). There have been several reports of both ACTH and cAMP displaying an antimitogenic effect on adrenocortical cells (Ramachandran & Suyama 1975, Hornsby & Gill 1977, Saez et al. 1981). However, mitogenic effects of both ACTH (Armato et al. 1977, Menapace et al. 1987, Arola et al. 1993a) and cAMP (Whitfield et al. 1976, Arola et al. 1993b) have also been demonstrated.

Protein kinase C (PKC) is known to play an important role in cell proliferation and differentiation processes (Nishizuka 1986). It has been suggested that ACTH regulates the intracellular distribution of PKC in the rat adrenal gland. After short-term ACTH treatment, a transfer of PKC from cytosol to membranes was reported in whole gland preparations (Farsee et al. 1987); following long-term ACTH administration, an increase in nuclear content but a decrease in cytosolic PKC content was found in zona glomerulosa (Lehoux et al. 1991). PKC has also been shown to participate in the aldosterone production of zona glomerulosa cells (Vinson et al. 1989, Kurscheid-Reich et al. 1992). A mitogenic effect caused by the activation of PKC with 12-O-tetradecanoyl phorbol-13-acetate (TPA) has been reported in rabbit (Menapace et al. 1987) and human (Mc Allister & Hornsby 1987) adrenocortical cells.

ACTH exerts a biphasic effect on the proliferation of rat adrenocortical cells in primary culture (Arola et al. 1993a). Undifferentiated, lipoprotein receptor-negative, zona
glomerulosa-like fetal rat adrenocortical cells differentiate into zona fasciculata-type cells during ACTH stimulation (Kahri 1966, Kahri et al. 1989). The primary antimitogenic effect after 24 h is followed by a highly stimulatory phase by the end of 72 h of ACTH treatment (Arola et al. 1993a). cAMP derivatives induce an ACTH-like effect on differentiation and steroidogenesis. 8-Bromo cAMP (8-Br cAMP) also has a stimulatory effect on proliferation after 72 h of treatment, but no antimitogenic effect is seen with 8-Br cAMP (Arola et al. 1993b). In the present paper, the roles of PKC and cAMP on ACTH-induced proliferation and differentiation are investigated. The parameters used to evaluate differentiation were ultrastructure, steroidogenic enzyme gene expression and steroid secretion. Protein kinase inhibitors staurosporine, H-7 and HA1004 and PKC activator TPA were used as tools with which to study the role of PKC. The cAMP derivative 8-Br cAMP was used to elucidate the role of cAMP.

Materials and Methods

Tissue culture

Tissue cultures previously shown to be suitable for long-term cultivation of fetal rat adrenals were used (Kahri 1966). Fetuses at 21 days were obtained from adult Sprague–Dawley rats, which were maintained under controlled lighting and temperature with free access to food pellets and tap water. Explants were derived from ten adrenals per culture dish. The culture medium (5 ml/dish) consisted of 50% Melnick’s solution A (Hanks’ balanced salt solution (BSS) plus 0.5% lacticalbumin hydrolysate), 25% Eagle’s minimum essential medium, and 25% heat-inactivated ultraltraltrated newborn calf serum (all from Gibco, Paisley, Strathclyde, UK). The medium was changed every 7 days. Experiments were performed after 21 days of cultivation, when the cortical cells cultured without ACTH or 8-Br cAMP appear as monolayer colonies of homogeneous undifferentiated zona glomerulosa-like cells (Kahri 1966), and lack binding sites for low-density lipoprotein (LDL) and high-density lipoprotein 3 (HDL3) (Kahri et al. 1989). ACTH (Cortrophine; Organon, Oss, The Netherlands) at a dose of 0.2 µg/ml (70 nmol/l) and 8-Br cAMP (Sigma Chemical Co., St Louis, MO, USA) at a dose of 35 µg/ml (80 µmol/l) were added to the cultures daily for 1–3 days. TPA at a dose of 20 ng/ml (30 nmol/l; Sigma) and staurosporine at a dose of 47 ng/ml (100 nmol/l; Boehringer Mannheim, Mannheim, Germany) were added once in experiments lasting 24 h or less. H-7 (1-(5-isoquinolinesulphonyl)-2-methylpiperazine dihydrochloride) at a dose of 18 µg/ml (50 µmol/l) and HA1004 (N-(2-guanidinoethyl)-5-isoquinolinesulphonamide hydrochloride) at a dose of 16 µg/ml (50 µmol/l) (both from Seikagaku Corporation, Tokyo, Japan) were added once in the proliferation experiments, but daily when steroid secretion was measured.

Immunocytochemical staining for cell proliferation

Incorporation of 5-bromo-2-deoxyuridine (BrdU; Sigma) into replicating DNA was used as an indicator of proliferation. [3H]BrdU has been found to be incorporated into nuclear DNA of rat adrenocortical cells with equal labelling indices when compared with tritiated thymidine (Kahri et al. 1976). Proliferating cells were demonstrated immunocytochemically in the cultures by using the cell proliferation kit (Amersham International plc, Amersham, Bucks, UK). BrdU is localized by a specific monoclonal antibody and stained by a peroxidase-based detection system (Gratzner et al. 1975, Gonchoroff et al. 1986). BrdU was added to the cultures at a dose of 20 µg/ml (50 µmol/l). After BrdU had been included for 15 h, the cultures were fixed with acetic acid (5%) and ethanol (95%) for 30 min and thereafter stained using the cell proliferation kit. The proliferation percentage was calculated from positive-staining nuclei. At least three monolayer colonies of fetal rat adrenocortical cells were photographed and counted from each culture dish, presenting a minimum of 5000 cells/dish. All the experiments were performed in triplicate dishes. Statistical evaluation of the data was performed by using unpaired two-tailed Student’s t-test.

RNA extraction and Northern analysis

For RNA analysis, monolayer colonies of adrenocortical cells were detached from the culture dishes under a phase-contrast microscope. The colonies were carefully cut from the surrounding fibroblasts and collected separately. Total cellular RNA was isolated by guanidium isothiocyanate extraction and cesium chloride centrifugation (Chirgwin et al. 1979). Extracted RNA was measured spectrophotometrically at 260 nm, and stored at −20 °C until use. Total RNA (8 µg) was denatured in glyoxal and dimethylsulphoxide, and loaded onto a 1.5% agarose gel. After electrophoresis, the RNA was transferred onto Hybond-N filter (Amersham) by capillary blotting (Thomas 1980). The filters were hybridized with rat cholesterol side-chain cleavage enzyme (P450sc) cRNA (65 °C, 16 h) and with mouse 28S cDNA (42 °C, 16 h) probes. The filters were subjected to autoradiography using Trimax T16 intensifying screens (3M, Ferrania, Italy) and Agfa Curix ST-L films (Agfa, Belgium) for 0–7 days at −70 °C. The intensities of the autoradiographic signals were quantitated by a densitometric scanner.

Probes

A rat P450sc riboprobe was kindly provided by S Townsend (University of Colorado, Denver, CO, USA)
(Townsend et al. 1990), and labelled with $[^{32}\text{P}]$UTP (1000 Ci/mmol; Amersham) by in vitro transcription using T7 RNA polymerase. Mouse 28S cDNA (Arnheim 1979) was used as a loading control. It was labelled with $[^{32}\text{P}]$dCTP by random priming (Oligolabelling Kit; Pharmacia, Uppsala, Sweden).

**Light and electron microscopy**

The viability of the cultures was followed by phase-contrast microscopy. For electron microscopy, the cultures were fixed in situ with 2.5% glutaraldehyde in Hanks’ BSS, postfixed in osmium tetroxide, dehydrated with a graded series of ethanol, and finally embedded in a mixture of Epon 812 (Shell, New York, NY, USA) and Araldite 6005 (Ciba, Basel, Switzerland).

**Steroid measurements**

Corticosterone (11-ß,21-dihydroxy-4-pregnene-3,20-dione) and 18-hydroxydeoxycorticosterone (18-OH-DOC, 18,21-dihydroxy-4-pregnene-3,20-dione) were measured as described previously (Salmenperä & Kahri 1976). The results were corrected for methodological losses as measured with a radioactive recovery indicator. Briefly, the method consists of dichloromethane extraction of the tissue culture medium and partition of the extracts between 80% aqueous ethanol and cyclohexane. The ethanolic extracts were purified using Sephadex LH-20 columns. Fractions containing corticosterone and 18-OH-DOC were collected. After gas chromatography on 1% SE-30 columns the steroids were quantified with a flame ionization detector as their O-methyloxime–trimethylsilyl–ether derivatives. In short-term experiments corticosterone was determined with radioimmunoassay (RIA), using rabbit anti-corticosterone (ICN Biomedicals, Costa Mesa, CA, USA) after extraction and purification of samples as described earlier (Salmenperä & Kahri 1976).

**Results**

**Proliferation of rat adrenocortical cells in vitro**

Fetal rat adrenocortical cells appeared as monolayer colonies of undifferentiated, zona glomerulosa-like cells, lacking the binding sites of fluorescent LDL and HDL-3, after 21 days of culturing in the absence of ACTH (Kahri 1966, Kahri et al. 1989). The percentage of the BrdU-positive nuclei was 10, and the level remained constant during the next 72 h of cultivation (Figs 1, 2 and 3). The biphasic effect of ACTH (daily dose of 70 nmol/l) on the proliferation of adrenocortical cells was also demonstrated. During the first 24 h of treatment, ACTH reduced the percentage of proliferating cells down to 2 (Fig. 2), but when stimulation was continued for 72 h, 23% of the cells stained BrdU-positive (Fig. 3).

PKC activator TPA at a dose of 30 nmol/l also had an inhibitory effect on the proliferation of rat adrenocortical cells. Stimulation with TPA for 24 h reduced the level of proliferating cells to 3% (Fig. 1). A combination of ACTH and TPA for 24 h inhibited the proliferation to the same extent as both substances alone (Fig. 2).

The role of PKC in the antimitogenic effect of ACTH was studied with the help of PKC inhibitors H-7 and staurosporine (Hidaka et al. 1984, Tamaoki et al. 1986). In addition to its effect on PKC, H-7 also inhibits protein kinase A (PKA). To ensure that the effects appearing with H-7 were due to the inhibition of PKC, HA1004 was used as a control for H-7. HA1004 is an equally potent PKA inhibitor as H-7, but it is only a weak inhibitor of PKC (Hidaka et al. 1984). H-7 and HA1004 both at the dose of 50 μmol/l or staurosporine at the dose 100 nmol/l had no effect on proliferation when added alone for 24 h: 12% of the H-7- and 10% of the HA1004- or staurosporine-treated adrenocortical cells stained BrdU-positive (Fig. 1). If H-7 or staurosporine were combined with ACTH during the first 24 h of treatment, the inhibitory effect of ACTH was almost totally blocked, and 9% of the nuclei were positive in both experiments.
stimulated

figure 2. The proliferation of fetal rat adrenocortical cells grown in the absence of ACTH for 21 days and thereafter stimulated with ACTH (70 nmol/l) or with combination of ACTH and staurosporine (Stauro; 100 nmol/l), H-7 (50 µmol/l), HA1004 (50 µmol/l) or 12-0-tetradecanoyl phorbol-13-acetate (TPA; 30 nmol/l) for 24 h, or left without treatment. The percentage of bromodeoxyuridine (BrdU)-positive nuclei was counted from at least three monolayer colonies of adrenocortical cells per dish, altogether a minimum of 5000 cells/culture dish. Means ± s.e.m. of triplicate culture dishes are presented; there were at least nine colonies in each group. *P<0.05 for ACTH, ACTH+H-7, ACTH+HA1004 and ACTH+TPA compared with control, and for ACTH+Stauro and ACTH+H-7 compared with ACTH (Student's t-test).

(2). However, a similar experiment with HA1004 did not prevent this ACTH-induced inhibition, and the proliferation rate was 3% (Fig. 2). The inhibitory effect of TPA was also reducible with H-7. Seven per cent of the nuclei stained positive with a combination of H-7 and TPA for 24 h (Fig. 1). This indicated that the inhibitory effect of TPA in this system was due to the activation of PKC.

We have previously demonstrated that cAMP derivatives 8-Br cAMP and dibutryl cAMP do not inhibit proliferation during 24-h stimulations, but treatment for 72 h with 8-Br cAMP stimulates proliferation in the same way as ACTH (Arola et al. 1993b). In the present study, stimulation with 8-Br cAMP for 72 h at a daily dose of 80 µmol/l raised the proliferation rate to 24%, which is 2.5-fold when compared with control cells, and equals the rate of ACTH-stimulated cells (Fig. 4).

The role of cAMP in the stimulatory growth effect of ACTH seems to be obvious. However, the possibility of an additional involvement of PKC was studied using staurosporine, H-7 and HA1004. All of them were combined with ACTH or 8-Br cAMP during the last 24 h of the 72-h stimulations. Thus, events occurring prior to the stimulatory growth effect were not disturbed.

staurosporine, and H-7 blocked the stimulatory effect of ACTH. When staurosporine or H-7 were combined with ACTH, the proliferation rate dropped from 23 to 12 and 9% respectively (Fig. 3) and a similar inhibition was detected with a combination of 8-Br cAMP and H-7 (Fig. 4). In contrast, HA1004 had no effect on ACTH-induced stimulation of proliferation (Fig. 3), and reduced 8-Br cAMP-induced proliferation only from 23 to 17% (Fig. 4).

Expression of P450<sub>sec</sub> in cultured fetal rat adrenocortical cells

The mRNA for P450<sub>sec</sub> was seen as a single band at about 2.0 kb in Northern blots prepared from cultured fetal rat adrenocortical cells, as described previously (Goldring et al. 1987). The effects of H-7 and TPA on P450<sub>sec</sub> mRNA were studied in control and ACTH-stimulated cultures by Northern blotting. Cultures were either stimulated with ACTH or left without it for 6–24 h. H-7 and TPA were used in both ACTH-treated and untreated cultures. Neither of the PKC regulators alone was able to increase the low control level of the P450<sub>sec</sub> mRNA, in fact H-7 even reduced the expression by 75%. In contrast, ACTH
increased the P450\textsubscript{sc} mRNA level twofold after 6 h and tenfold after 24 h. H-7 decreased this ACTH-induced gene expression by 75 and 50% after 6 and 24 h respectively. However, TPA had no effect on ACTH-induced levels of P450\textsubscript{sc} (Fig. 5).

Steroid secretion

The concentrations of corticosterone and 18-OH-DOC in the culture medium were measured. ACTH increased steroid secretion 20-fold when stimulations were continued for 24–72 h. H-7 and TPA alone did not increase the steroid secretion (Fig. 6). If H-7 was combined with ACTH for 24 h, 70% of the corticosterone secretion was reduced (Fig. 6). However, if the combination of ACTH and H-7 was continued for 72 h, the reduction of steroid secretion was no longer noticeable with H-7 (Fig. 7). Similar experiments performed with HA1004 did not inhibit the ACTH-induced steroid secretion (data not shown). Neither was any change in the steroids seen if TPA was combined with ACTH for 24 h (Fig. 6). The increase of corticosterone secretion with 8-Br cAMP was seven- and tenfold after 24 and 72 h respectively. H-7 did not inhibit these secretions. However, TPA in combination with 8-Br cAMP for 24 h doubled the corticosterone secretion, when compared with 8-Br cAMP alone (Fig. 6).

**Differentiation of fetal rat adrenocortical cells**

Ultrastructurally, unstimulated rat adrenocortical cells appeared as undifferentiated zona glomerulosa-like cells and differentiated into zona fasciculata-type cells when stimulated with ACTH for 72 h. The shape of the mitochondrial inner membrane transformed from lamellar to vesicular, and the amount of smooth endoplasmic reticulum increased (Kahri 1966, 1968). These changes were also detected even if the cultures were simultaneously treated with H-7, HA1004 or TPA. However, cultures treated with TPA alone possessed features typical of zona glomerulosa cells.

**Discussion**

ACTH stimulates the growth of the adrenal gland in vivo. An elevated concentration of ACTH causes enlargement of the gland and increases the synthesis and content of DNA in adrenal cortex (Liddle et al. 1962, Farese & Reddy 1963, Masui & Garren 1970). ACTH induces differentiation of zona glomerulosa cells into zona...
fasciculata cells in vivo and in vitro (Sabatini et al. 1962, Kahri 1966). However, the proliferative activity varies in different zones. Mitotically active regions are zonae glomerulosa and outer fasciculata, whereas inactive areas are inner fasciculata and reticularis (Mitchell 1948, Diderholm & Hellman 1960, Walker & Rennels 1961, Ford & Young 1963, Wright 1971). There is also a narrow area between the glomerulosa and fasciculata, called zona intermedia, which lacks proliferative activity (Mitchell 1948). Thus, depending on the stage of differentiation, the proliferation of adrenocortical cells is either stimulated or inhibited. Reports from in vivo studies concerning the proliferative effects of ACTH have varied from antimitogenic (Masui & Garren 1971, Ramachandran & Suyama 1975, Hornsby & Gill 1977, Salmenperä & Kahri 1977) to mitogenic (Armato & Nussdorfer 1972, Armato et al. 1977, Menapace et al. 1987). The discrepancy in the results may be due to the varying stage of differentiation of the adrenocortical cells. The cultured fetal rat adrenocortical cells used in our study possess parameters typical of zona glomerulosa after 21 days of culturing in the absence of ACTH, and when treated with ACTH for 72 h they present features typical for fasciculata cells (Kahri 1966, Kahri et al. 1970, 1989, Fong et al. 1989). ACTH induces a biphasic proliferation curve on these cells, having an inhibitory effect during the first 24 h but a stimulatory effect by the end of 72 h (Arola et al. 1993a).

cAMP is known to play a key role in the transduction system leading to differentiation of the adrenocortical cells (Nussdorfer et al. 1973, Arola et al. 1993b). There are numerous reports on the antimitogenic effect of cAMP (Ramachandran & Suyama 1975, Hornsby & Gill 1977, Saez et al. 1981). However, in many different cell systems cAMP has been found to mediate growth-promoting
factors (Dumont et al. 1989), and also rat and human adrenocortical cells have been shown to increase their proliferative activity after cAMP treatment (Whitfield et al. 1976, Boynton & Whitfield 1983). The antimitogenic effect of ACTH on fetal rat adrenocortical cells is independent of cAMP, since cAMP has only a mitogenic effect on these cells (Arola et al. 1993b).

PKC, an important transducer of cell proliferation and differentiation in many different cell types (Nishizuka 1986), is also triggered by ACTH (Farre et al. 1987, Lehoux et al. 1991). It has been suggested that PKC takes part in human fetal adrenal growth as a cAMP-independent mechanism (Pepe & Albrecht 1990). In the present study TPA, an activator of PKC, was antimitogenic for rat adrenocortical cells. This effect could be prevented with the PKC inhibitor H-7. In a similar fashion, the antimitogenic effect of ACTH was inhibited by H-7 or staurosporine but was unaffected by HA1004, a protein kinase inhibitor with greater activity upon PKA. These results suggest that the primary inhibitory growth effect of ACTH is transduced via PKC.

Expression of mRNAs for steroidogenic enzymes and increased steroid hormone production have been shown to be transduced via cAMP (Ramachandran & Suyama 1975, Hornsby & Gill 1977, Sacz et al. 1981, Voutilainen & Miller 1987). It has been shown that PKC participates in the aldosterone production of the zona glomerulosa (Vinson et al. 1989, Elliot et al. 1991, Kurscheid-Reich et al. 1992). The role of PKC in the secretion of steroid hormones by the glomerulosa-type cells was also demonstrated in our study. H-7 inhibited ACTH-induced corticosterone secretion by the glomerulosa-type cells. 8-Br cAMP-stimulated cells secreted less corticosterone than did ACTH-treated cells after 24 h, but this secretion was doubled by TPA and unaffected by H-7 treatment. TPA alone, however, was not able to induce steroid production, neither was it able to increase the steroids produced by ACTH further. When stimulations with 8-Br cAMP or ACTH were continued for 72 h, H-7 no longer inhibited steroid production. Thus, both cAMP and PKC are involved in the steroidogenic action of ACTH in the glomerulosa. cAMP alone can induce steroid production, but PKC alone is not capable of so doing. However, continuous steroid production of the fasciculata-type cells is a PKC-independent phenomenon.

PKC also appears to have some role in ACTH-induced P450scc mRNA expression by undifferentiated zona glomerulosa-type rat adrenocortical cells. Inhibition of PKC by H-7 dramatically reduces the expression of the P450scc gene after 6 and 24 h of ACTH stimulation. However, activation of PKC with TPA alone cannot increase the expression of this gene. cAMP derivatives have been shown to induce the ACTH-like expression of the P450scc mRNA in man (Ilvesmäki & Voutilainen 1991) and rats (Arola et al. 1993b). It has been suggested that an additional cAMP-independent second messenger exists in P450scc gene regulation in cultured bovine adrenocortical cells (Hanukoglu et al. 1990). Data presented here suggest that PKC together with cAMP can be involved in the regulation of P450scc gene expression of fetal rat adrenocortical cells.

Parallel activation of PKA and PKC also appears to be possible in the mitogenic effect of ACTH. Although an ACTH-like mitogenic phase can be achieved with cAMP derivatives, both ACTH- and 8-Br cAMP-induced activations of proliferation can be prevented with H-7 or staurosporine but not with HA1004. The interaction of these two different signal transduction pathways is obvious. The involvement of cAMP and PKC in the mitogenic effect of ACTH on rabbit adrenocortical cells has been suggested (Menapace et al. 1987). It has been found that thyrotropin- or insulin-like growth factor-I-induced proliferation of rat FRTL-5 thyroid cells is mediated by two different signal transduction pathways, PKC and cAMP (Fujimoto & Brenner-Gati 1992). Nishizuka (1986) has proposed a model of interaction of two signal transduction systems, where in endocrine tissues cAMP-PKA would stimulate the diacylglycerol-PKC pathway. Another possibility could be an additional transcription factor like activator protein 2, which mediates induction by two different signal-transduction pathways, namely PKC and cAMP (Imagawa et al. 1987). Two different actions of PKC, i.e. growth inhibitory for glomerulosa-type cells and stimulatory for fasciculata-type cells, seem to be confusing. However, a family of different PKC isoenzymes exists, and their expression pattern is cell-type and differentiation-stage specific. Distinct PKC isoenzymes may activate different cellular pathways and phosphorylate different substrates (Azzi et al. 1992).

Adrenocortical cells stimulated with either ACTH or cAMP derivatives differentiate from zona glomerulosa-like cells into fasciculata-type cells (Kahri 1966, Nussdorfer et al. 1973). PKC regulators do not affect this process. Activation of PKC alone by TPA does not lead to any morphological changes. Thus, differentiation of glomerulosa-like fetal rat adrenocortical cells into fasciculata-like cells is transduced only via cAMP and does not require the involvement of PKC.

In conclusion, our results suggest that both cAMP-PKA and PKC are involved in the signal transduction of the biphasic growth effects of ACTH on fetal rat adrenocortical cells. PKC plays a role in the antimitogenic effect of ACTH, and both cAMP and PKC are involved in the stimulatory growth phase induced by ACTH. Differentiation of adrenocortical cells and the steroid production of fasciculata-type cells are transduced via cAMP.

Acknowledgements

The skilful technical assistance of Eija Heiliö, Merja Haukka and Päivi Laitinen is gratefully acknowledged.
We also thank Dr Susan Townsend (Department of Pediatrics, University of Colorado, Denver, CO, USA) for the rat P450c cRNA probe. This work was financially supported by the Academy of Finland and Sigrid Juselius Foundation (to R.V).

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Received 20 August 1993
Accepted 9 December 1993