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Cell Biology

Angiotensin stimulation of bovine adrenocortical cell growth

(fibroblast growth factor/insulin/angiotensin antagonist/corticotropin)

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ABSTRACT **Factors controlling proliferation of adrenocortical cells have been studied in monolayer cultures of bovine adrenocortical cells. Angiotensin II stimulated cell proliferation and [^{3}H]thymidine incorporation into DNA with a half-maximal effective concentration of 0.96 ± 0.27 nM. Similar sensitivity to angiotensin III with reduced sensitivity to angiotensin I and tetradecapeptide renin substrate was observed. Although sensitivity to angiotensin II was equivalent to that for fibroblast growth factor (1.5 nM half-maximal effective concentration), maximal effects of angiotensin were less than for fibroblast growth factor and serum. High concentrations of insulin (1–10 μM) also stimulated [^{3}H]thymidine incorporation into DNA and cell proliferation. [Sar^1,Ile^5,Ile^8]Angiotensin II, a competitive antagonist of angiotensin II, blocked angiotensin II stimulation of DNA synthesis but did not affect fibroblast growth factor and insulin stimulation of DNA synthesis. Corticotropin (ACTH) blocked the stimulatory effects of both angiotensin II and fibroblast growth factor. The dose–response curves for angiotensin II stimulation of steroidogenesis were similar to those for stimulation of [^{3}H]thymidine incorporation into DNA. Among the seven cell types examined, only adrenocortical cells responded to angiotension II with stimulation of DNA synthesis.**

Although several purified polypeptides have been reported to stimulate the proliferation of animal cells in culture, a high degree of cell specificity has not been observed. Fibroblast growth factor (FGF) is mitogenic for fibroblast, adrenocortical, myoblast, smooth muscle, chondrocyte, vascular endothelial, granulosa, and luteal cells, whereas epidermal growth factor (EGF) is mitogenic for fibroblast, corneal epithelial, certain mammary epithelial, and granulosa cells (1). Several pituitary polypeptides long considered trophic for specific target cells *in vivo* either inhibit DNA synthesis when added directly to target cells in culture or have no effect on growth (2–6). Understanding of the mechanisms controlling organ-specific growth is, therefore, incomplete; interaction of several growth factors and hormones may be involved (7).

A recently developed strain of bovine adrenocortical cells in culture has been used to study factors controlling proliferation of the adrenal cortical cell (8). FGF but not EGF stimulates proliferation of these cells, whereas corticotropin (ACTH) and prostaglandin E_1 (PGE_1), which stimulate cyclic AMP formation, inhibit proliferation (8, 9). Corticotropin and PGE_1 both stimulate steroidogenesis and induce the steroid biosynthetic pathway. The present studies indicate that angiotensin II and III at low concentrations stimulate DNA synthesis as well as steroidogenesis in adrenocortical cells. The growth-stimulatory effect of angiotensin appears specific because several cell types, including cells reported to possess angiotensin II receptors, do not increase DNA synthesis with addition of angiotensin II or III.

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METHODS AND MATERIALS

Growth of Cells. Primary bovine adrenocortical cells were routinely grown in Coon's modification of Ham's F-12 medium with 10% horse serum (Colorado Serum Co.) and 2.5% fetal calf serum (Irvine) (8). Purity of the adrenocortical cell cultures has been documented by uniform inhibition of cell replication by corticotropin (8, 9) and by continuous proliferation of a uniform cell type for over 50 generations in culture (10). Cell growth was arrested by removal of serum and incubation in medium alone for 72 hr. Flow microfluorometric analysis indicated arrest in G_1. A cloned line prepared by the procedure of Puck *et al.* (11) and designated AC1 was grown under identical conditions. Bovine granulosa cells prepared as described by Gospodarowicz *et al.* (6) were maintained in Coon's F-12 containing 10% horse serum; cell growth was arrested by incubation in medium alone for 48 hr. Bovine aortic endothelial cells and bovine vascular smooth muscle cells were provided by D. Gospodarowicz (12). Cells were maintained in Dulbecco's modified Eagle's medium with 10% calf serum; growth was arrested by incubation in Dulbecco's modified Eagle's medium containing 0.2% calf serum for 48 hr. Primary cultures of bovine lung fibroblasts were prepared by the same procedure as bovine adrenocortical cells. Cells were maintained in Coon's F-12 containing 10% horse serum; cell growth was arrested by incubation in medium alone for 48 hr. BALB/c 3T3 cells, provided by R. Holley, were grown in Coon's F-12 containing 10% horse serum; growth was arrested by incubation in medium containing 0.5% horse serum for 48 hr. Rabbit chondrocytes were prepared as described by Ham and Sattler (13). Cells were maintained in Coon's F-12 containing 10% horse serum; growth was arrested by incubation in medium alone for 72 hr.

Measurement of Cell Growth and of [^{3}H]Thymidine Incorporation. For determination of cell number medium was removed and cells were detached by incubation with 1 ml of Tris-buffered saline (14) containing 0.05% trypsin and 5 mM EDTA for 10 min at room temperature. Cells were diluted and counted immediately in a model Z_F Coulter Counter. For quantitation of DNA synthesis [*methyl*-^{3}H]thymidine (60 Ci/mmol, 10 μCi per plate) (Schwarz/Mann) was routinely added to cultures 12 hr after addition of growth stimuli. Twelve hours later, medium was removed and 1 ml of a 1% aqueous solution of Triton X-100 was added. The cells were incubated with this solution for 5 min and the entire contents of the plate

Abbreviations: FGF, fibroblast growth factor; EGF, epidermal growth factor; PGE_1, prostaglandin E_1; renin substrate, tetradecapeptide H_2N-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser-OH; angiotensin I, decapeptide H_2N-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-OH; angiotensin II, octapeptide H_2N-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH; angiotensin III, des-Asp^1-angiotensin II; angiotensin II antagonist, [Sar^1,Ile^5,Ile^8]angiotensin II; renin inhibitor, H_2N-His-Pro-Phe-His-Leu-DLeu-Val-Tyr-OH; angiotensin-converting enzyme inhibitor, <Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro-OH; ED_{50}, half-maximal effective concentration.

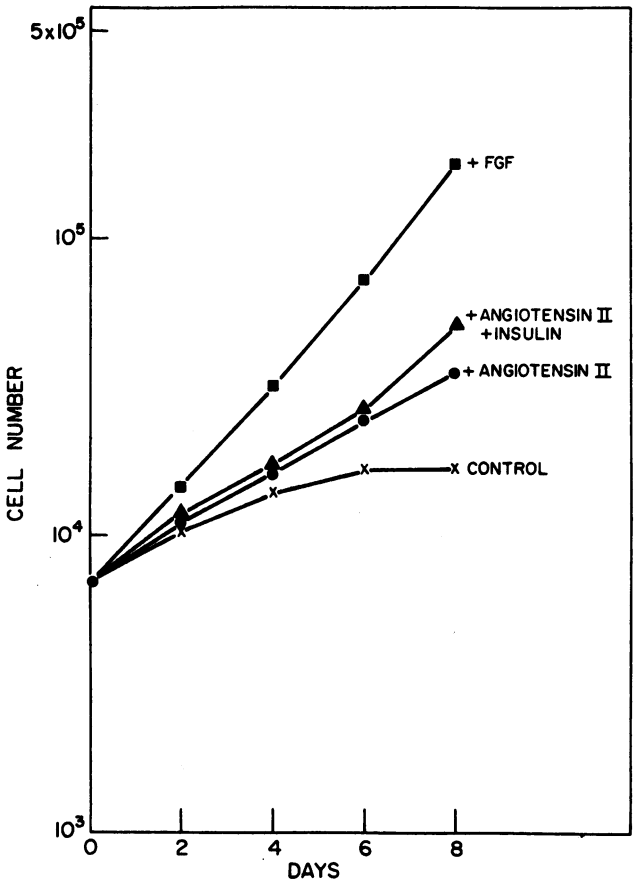

FIG. 1. Effect of FGF, angiotensin II, and insulin plus angiotensin II on bovine adrenocortical cell proliferation. Second passage bovine adrenocortical cells were subcultured into 3.5-cm dishes with Coon's F-12 medium and 10% horse serum. Twelve hours later, medium was removed and replenished with Coon's F-12 containing 1% horse serum (×) with angiotensin II (10 ng/ml) (●), angiotensin II (10 ng/ml) plus insulin (1 μg/ml) (▲), or FGF (50 ng/ml) (■). Media with fresh additions were replaced every 2 days and cell number on duplicate plates was determined.

were transferred to 10 ml of absolute ethanol. This material was filtered under vacuum through 2.4-cm glass fiber filters (GF/A, Whatman), and the filters were washed twice with 10 ml of absolute ethanol and assayed for radioactivity in toluene/Liquifluor (New England Nuclear) (3.8 liters/180 ml). Preliminary experiments indicated G_1 growth arrest in serum-free medium with the initiation of DNA synthesis occurring 8–10 hr after re-addition of growth stimuli.

Fluorogenic Steroid Production. After incubation with the indicated additions, medium was removed and extracted with dichloromethane. Fluorogenic steroid content was quantitated by ethanolic sulfuric acid fluorescence (15). Because cortisol and corticosterone are the principal fluorogenic steroids produced by bovine adrenocortical cells (16), results are expressed as μg of cortisol equivalent.

Materials. Purified FGF was a gift from Denis Gospodarowicz (17). Renin substrate, angiotensins I, II, and III, [Sar[1], Ile[5],Ile[8]]angiotensin II, renin inhibitor, angiotensin-converting enzyme inhibitor, bradykinin, and arginine vasopressin were obtained from Bachem Inc.; angiotensin II was also obtained from Schwarz/Mann, Vega Fox, and Calbiochem., and as a gift from Morton Printz. Corticotropin-(1–39)-nonatriacontapeptide was obtained from Armour Pharmaceutical; crystalline bovine insulin, serotonin, and histamine were from Sigma; tripeptide Gly-His-Lys was obtained from Pierce Biochemicals. Bovine

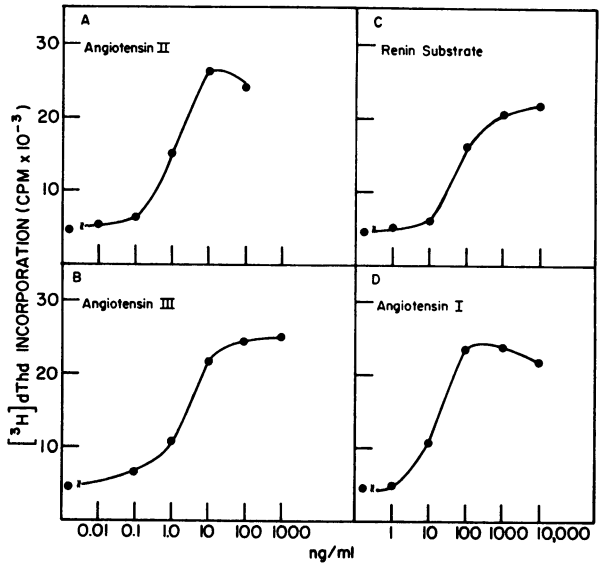

FIG. 2. Effect of increasing concentrations of angiotensin on [³H]thymidine incorporation into DNA in bovine adrenocortical cells. Cells were subcultured at 50,000 cells per 3.5-cm dish in Coon's F-12 medium containing 10% horse serum. Twelve hours later, medium was removed and cells were incubated for 72 hr in medium without serum. Various concentrations of the indicated substances were then added. Twelve hours later, [³H]thymidine (60 Ci/mmol, 10 μCi per plate) was added; after an additional 12-hr incubation, [³H]thymidine incorporation into DNA was determined. (*A*) angiotensin II; (*B*) angiotensin III; (*C*) renin substrate; (*D*) angiotensin I.

prolactin NIH-P-B4 and porcine growth hormone lot 400864 were obtained through the pituitary hormone distribution program, National Institute of Arthritis, Metabolism, and Digestive Diseases. β-Lipotropin, α, β, and γ endorphins, and corticotropin-(18–39)-docosapeptide were gifts from Roger Guillemin.

RESULTS

Effect of Angiotensin II, Insulin, and FGF on Cell Growth. Bovine adrenocortical cells maintain the cell-specific function of steroidogenesis through their life span of 55–65 generations in culture (10). Corticotropin, PGE_1, cholera toxin, and monobutyryl cyclic AMP stimulate the rate of steroid hormone production approximately 10-fold. These same substances that increase the rate of cyclic AMP formation inhibit DNA synthesis and cell replication. Adrenocortical cell growth was stimulated by serum and FGF but not by EGF (8). As shown in Fig. 1, angiotensin II also stimulated the growth of bovine adrenocortical cells in culture. Insulin, which has been reported to stimulate rat adrenocortical cell growth (3), was also active when added in high concentrations, but both angiotensin II and insulin were less potent than FGF in stimulating bovine adrenocortical cell growth. As previously reported for FGF (8), sustained cell growth required serum in addition to angiotensin II or insulin. In the absence of serum, FGF, angiotensin II, and insulin stimulated DNA synthesis but were incapable of supporting sustained cell proliferation.

Characterization of Angiotensin Stimulation of DNA Synthesis. The effect of increasing concentrations of angiotensin on DNA synthesis in G_1-arrested bovine adrenocortical cells is shown in Fig. 2. Angiotensin II stimulated [³H]thymidine incorporation into DNA with a half-maximal effect at 0.96 ± 0.27 nM ($n = 4$) and a maximal effect at 10 nM. In contrast to vascular tissue, which responds specifically to angiotensin II (18), adrenocortical cells respond to both angiotensin II and III

Table 1. Effectiveness of angiotensins in stimulating DNA synthesis in bovine adrenocortical cells

Material	ED_{50}, nM	[^{3}H]Thymidine incorporation at maximum, cpm
Angiotensin II	0.96 ± 0.27	24,431 ± 1,758
Angiotensin III	3.4	24,439 ± 1,789
Angiotensin I	26.7	23,843 ± 642
Renin substrate	37.3	20,602 ± 991

Half-maximal effective concentrations are averages derived from dose–response curves performed on two separate occasions; the value for angiotensin II is derived from four separate experiments (mean ± SD). Maximal incorporation was determined from triplicate plates in a single experiment. Incorporation in control culture plates was 4605 ± 594 cpm.

by increasing aldosterone synthesis (19). Angiotensin III also stimulated DNA synthesis in bovine adrenocortical cell cultures (Fig. 2*B*). Half-maximal stimulatory effects were noted at 3.4 nM angiotensin III. At high concentrations tetradecapeptide renin substrate and decapeptide angiotensin I stimulated DNA synthesis with half-maximal effective concentrations (ED_{50}s) of 37.3 and 26.7 nM, respectively (Fig. 2 *C* and *D*). Although the dose–response curves for the angiotensin II precursors, renin substrate and angiotensin I, were shifted to the right of those for angiotensin II and III by more than one order of magnitude, similar maximal effects were observed (Table 1). The stimulatory effects of renin substrate and angiotensin I appeared to be direct rather than through conversion to angiotensin II. When an inhibitor of renin activity or an inhibitor of angiotensin-converting enzyme activity was added with renin substrate, no change in the ability of renin substrate to stimulate [^{3}H]thymidine incorporation into DNA was noted. When angiotensin-converting enzyme inhibitor was added with angiotensin I, a 55% decrease in the activity of angiotensin I was noted, indicating that partial conversion to angiotensin II occurred. Higher concentrations of converting enzyme inhibitor (up to 10 μg/ml) gave no further inhibition, indicating that the effects of angiotensin I were in part direct.

The sensitivity of bovine adrenocortical cells to angiotensin II was equivalent to sensitivity to FGF [ED_{50} for angiotensin II = 0.96 nM versus ED_{50} for FGF = 1.5 nM (8)]. However, angiotensin II in maximal concentrations was less effective than FGF in stimulating bovine adrenocortical cell DNA synthesis and growth (Table 2 and Fig. 1). Under serum-free conditions, FGF was slightly less effective than 10% horse serum in stimulating DNA synthesis. Insulin also stimulated DNA synthesis in bovine adrenocortical cells (Table 2). When increasing concentrations of insulin were added, linearly increasing stimulation occurred from 10 to 10,000 ng/ml. Insulin sensitivity was much less than angiotensin II and FGF sensitivity. [^{3}H]Thymidine incorporation with insulin at 10 μg/ml (1.75 μM) was equivalent to 65% of that observed with angiotensin II at 10 ng/ml (9.6 nM). When insulin and angiotensin II were added together, synergism occurred, with incorporation equivalent to that obtained with FGF (Table 2). Insulin and angiotensin II were, however, less effective than FGF in stimulating cell proliferation (Fig. 1). Angiotensin II was additive with submaximal concentrations of FGF but the maximal incorporation was only equivalent to maximal FGF effects. Although submaximal concentrations of angiotensins II and III were additive, maximal concentrations of both were only equal to the maximal concentration of either. As previously reported for both serum and FGF stimulation of DNA synthesis and cell growth (8, 9), corticotropin blocked angiotensin II stimulation of DNA synthesis (Table 2). Several other substances were tested

Table 2. Comparison of stimuli of DNA synthesis in bovine adrenocortical cells

Additions	Concentration, ng/ml	[^{3}H]Thymidine incorporation, cpm
None		4,605 ± 594
Insulin	100	9,298 ± 919
Insulin	1,000	13,465 ± 586
Insulin	10,000	16,572 ± 172
Angiotensin II	10	24,431 ± 1,758
Angiotensin II + III	10 + 10	25,632 ± 2,500
FGF	50	43,513 ± 2,110
FGF	100	59,505 ± 4,776
Horse serum	10%	76,605 ± 4,030
Insulin + angiotensin II	1000 + 10	56,109 ± 3,948
FGF + angiotensin II	50 + 10	52,383 ± 1,885
FGF + angiotensin II	100 + 10	55,829 ± 257
Corticotropin + angiotensin II	4500 + 10	5,345 ± 318

Cell growth was arrested by incubation in Coon's F-12 medium without serum for 72 hr. Indicated additions were then made and [^{3}H]thymidine incorporation into DNA was determined.

both alone and with angiotensin II for their ability to stimulate DNA synthesis in bovine adrenocortical cells. The following substances were inactive: bovine prolactin, porcine growth hormone, vasopressin, tripeptide Gly-His-Lys that was reported to stimulate hepatic cell growth (20), luteinizing hormone-releasing hormone, bradykinin, histamine, serotonin, corticotropin-(18–39), β-lipotropin, and α, β, and γ endorphins.

Separation of Stimulatory Effects of Angiotensin II from Those of FGF and Insulin. In order to determine whether FGF, insulin and angiotensin II stimulate bovine adrenocortical cell DNA synthesis through occupancy of similar or different receptor sites, [Sar^1,Ile^5,Ile^8]angiotensin II, a competitive inhibitor of angiotensin II stimulation of vascular contractility (21) and adrenal aldosterone synthesis (19), was added along with FGF, insulin, or angiotensin II. As shown in Fig. 3, progressive inhibition of angiotensin II stimulation of DNA synthesis occurred. [Sar^1,Ile^5,Ile^8]Angiotensin II had no inhibitory

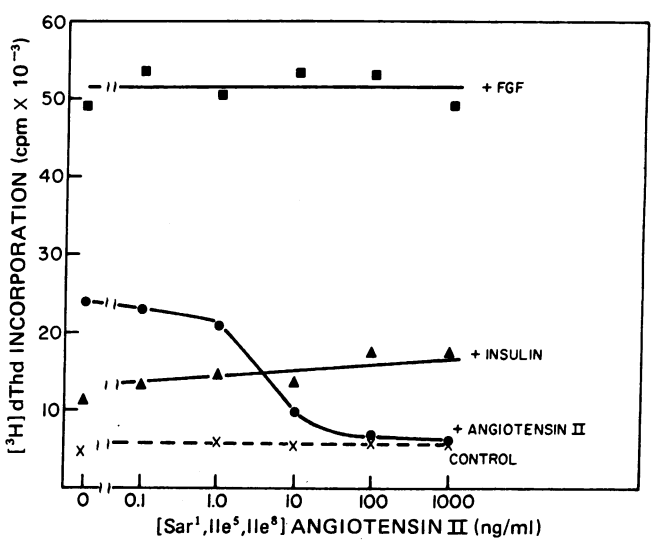

FIG. 3. Effect of [Sar^1,Ile^5,Ile^8]angiotensin II on angiotensin II-, FGF-, and insulin-stimulated [^{3}H]thymidine incorporation into DNA. G_1-arrested cell cultures were prepared as indicated in Fig. 2. Various concentrations of [Sar^1,Ile^5,Ile^8]angiotensin II were added without (×) or with angiotensin II (10 ng/ml) (●), FGF (50 ng/ml) (■), or insulin (10 μg/ml) (▲), and [^{3}H]thymidine incorporation into DNA was determined as described in Fig. 2.

Table 3. Cellular specificity of angiotensin II stimulation of DNA synthesis

Cell type	[^{3}H]Thymidine incorporation, stimulated/control		
	Serum, 10%	FGF, 100 ng/ml	Angiotensin II, 100 ng/ml
Bovine granulosa	12.3	5.5	1.3
Bovine aortic endothelial	4.6	2.3	1.0
Bovine vascular smooth muscle	77.4	11.8	1.0
Bovine lung fibroblasts	26.5	10.1	1.0
BALB/c 3T3	143.5	44.5	1.0
Rabbit ear chrondrocytes	65.0	46.0	1.0
AC1 bovine adrenocortical	20.4	24.9	16.4

Stock cultures of the various cells were grown as described under *Materials and Methods*. Cells were plated at ~10^5 cells per 3.5-cm dish and growth was arrested by either removal of serum or incubation in reduced serum as described for each cell type under *Materials and Methods*. The indicated additions were made without medium change at time 0; 12 hr later, [^{3}H]thymidine (60 Ci/mmol, 10 μCi per plate) was added; after an additional 12 hr, cells were analyzed for [^{3}H]-thymidine incorporation into DNA. Incorporation in duplicate control cultures was as follows: bovine granulosa cells, 37,503 cpm; bovine aortic endothelial cells, 5787 cpm; bovine vascular smooth muscle cells, 1290 cpm; bovine lung fibroblasts, 896 cpm; BALB/c 3T3 cells, 3767 cpm; rabbit ear chondrocytes, 1605 cpm; and AC1 bovine adrenocortical cells, 3728 cpm. In all cases, angiotensin II was tested over the dose range of 0.01–1000 ng/ml. The maximal response is shown. Because serum is required for optimal mitogenic effects of FGF in several of the cell types used, the FGF responsiveness observed represents a minimal value.

effect on FGF or insulin-stimulated DNA synthesis, indicating that different receptor sites were involved in the action of angiotensin II and FGF or insulin. The slight stimulatory effect of [Sar^1,Ile^5,Ile^8]angiotensin II alone was more evident in the presence of insulin. [Sar^1,Ile^5,Ile^8]Angiotensin II at high concentrations (up to 10 μg/ml) failed to inhibit serum-stimulated DNA synthesis (data not shown).

Cell Specificity. Although FGF effects are noted in many cell types, the growth-stimulatory effects of angiotensin II appear relatively specific (Table 3). Bovine granulosa cells, which are of similar embryological origin as adrenocortical cells (22), do not respond to angiotensin II. Bovine aortic endothelial and bovine vascular smooth muscle cells, which are reported to respond to angiotensin II with synthesis of prostaglandins (D. Gospodarowicz, personal communcation), also failed to increase DNA synthesis in response to angiotensin II addition. Other bovine cells such as lung fibroblasts were similarly unresponsive. A clonal line derived from the bovine adrenocortical cells responded to angiotensin II at an ED_{50} of 0.66 ± 0.34 nM (n = 4) similar to the responsiveness of the uncloned strain used in the present studies.

Effect of Angiotensin II on Steroidogenesis. In order to quantitate the effect of angiotensin II on the differentiated function of steroidogenesis, various concentrations of angiotensin II were added to confluent cultures and fluorogenic steroid products quantitated after a 2-hr incubation. Angiotensin II stimulated fluorogenic steroid production 2.7-fold with an ED_{50} of 0.32 nM (Fig. 4). Under the incubation conditions used, angiotensin II was as effective as corticotropin. Because angiotensin II is rapidly degraded, rates of steroid production

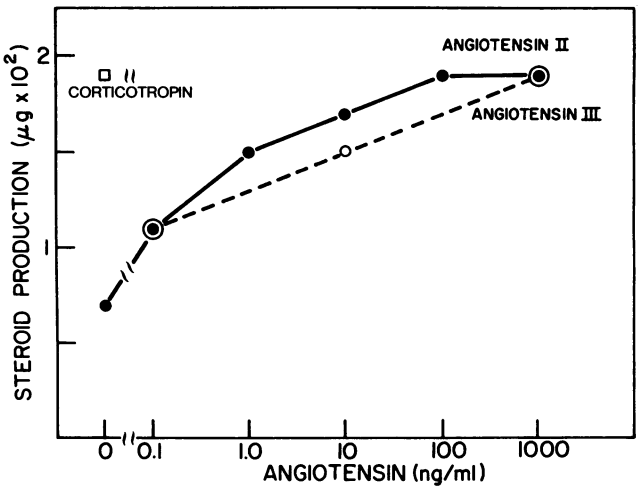

FIG. 4. Effect of angiotensin II and III on fluorogenic steroid production in bovine adrenocortical cells. Second passage bovine adrenocortical cells were subcultured at 10^5 cells per 3.5-cm dish in Coon's F-12 medium with 10% fetal calf serum plus FGF (100 ng/ml). Confluent cultures (6 × 10^4 cells per cm^2) were incubated with 5% fetal calf serum for 2 hr to remove steroids present and then placed in medium containing bovine serum albumin (1 mg/ml). The indicated concentrations of angiotensin II (●), angiotensin III (○), or corticotropin (1 μM) (□) were added for 2 hr and fluorogenic steroid production in duplicate plates was quantitated.

in response to angiotensin II were linear only during short incubations without or with low amounts of serum. Corticotropin stimulated steroidogenesis over prolonged periods even in the presence of serum. Angiotensin III was as effective as angiotensin II.

DISCUSSION

The apparent affinity and structural requirements for angiotensin stimulation of DNA synthesis resemble those reported for angiotensin binding to bovine adrenocortical cell membrane receptors (23). The equilibrium association constant of radiolabeled angiotensin II binding to bovine adrenocortical receptors of 0.5 nM^{-1} at 22° agrees well with the half-maximal concentration of angiotensin II of 0.96 ± 0.27 nM required for stimulation of [^{3}H]thymidine incorporation into DNA. Direct binding studies demonstrated that angiotensin III was only slightly less effective than angiotensin II, while tetradecapeptide renin substrate and angiotensin I were less potent by 1–2 orders of magnitude (23). The same relative affinities were evident for angiotensin stimulation of DNA synthesis. Moreover, [Sar^1,Ile^5,Ile^8]angiotensin II, a competitive inhibitor of angiotensin II binding to receptors and of angiotensin II stimulation of aldosterone production, blocked angiotensin II stimulation of DNA synthesis, indicating that the same structure–activity relationships exist for both activities of angiotensin II. The dose–response curves for angiotensin II stimulation of fluorogenic steroid production in these cells were similar to those for stimulation of [^{3}H]thymidine incorporation into DNA. Although the ED_{50} for angiotensin II stimulation of DNA synthesis and fluorogenic steroid production agrees well with the equilibrium association constant for angiotensin II binding in bovine adrenocortical cells, these affinities are low compared to the reported circulating plasma levels of angiotensin II (about 0.01 nM) and the ED_{50} for stimulation of aldosterone synthesis in canine glomerulosa cells (24, 25). Binding sites that appear "spare" for aldosterone synthesis in canine cells are more directly related to stimulation of steroid production and DNA synthesis in cultured bovine adrenocortical cells.

Although low concentrations of angiotensin II stimulate [^{3}H]thymidine incorporation into DNA in the absence of serum, the maximal effect is significantly less than that observed with FGF or with serum. In the presence of low concentrations of serum, angiotensin II remains less effective than FGF in stimulating cell proliferation. Although serum is required for angiotensin stimulation of proliferation, angiotensin is rapidly degraded by serum, so that effects on cell proliferation may be underestimated. In analogy with the studies of Hayashi and Sato (7), additional factors appear required to stimulate adrenocortical cell proliferation equivalent to that seen with serum. Insulin, which at high concentrations stimulates [^{3}H]thymidine incorporation into DNA, is more than additive with angiotensin II. Insulin and angiotensin II, however, remain less effective than serum, suggesting that additional factors are required to sustain proliferation.

Angiotensin stimulation of DNA synthesis appears relatively specific for adrenocortical cells. Among the cells tested by DNA synthesis initiation assays, only the adrenocortical cell strain and a derived clone responded. Although vascular cells are the other major target for angiotensin (24) and although biochemical responses to angiotensin have been shown in both smooth muscle and endothelial cells (26, 27), no effect on DNA synthesis was observed. Although Ganten *et al.* (28) indicated that high concentrations of angiotensin II (0.5–50 μg/ml) stimulated 3T3 cell growth and that high concentrations of the angiotensin inhibitor [Sar^1,Ala^8]angiotensin II (5 μg/ml) inhibited serum-stimulated growth of both 3T3 and SV3T3 cells, the present studies were unable to demonstrate an effect of angiotensin II or angiotensin II antagonists on DNA synthesis in 3T3 cells (data not shown). Moreover, [Sar^1,Ile^5,Ile^8]angiotensin II failed to block serum-stimulated DNA synthesis or growth of bovine adrenocortical cells. No explanation for the relative cell specificity of the growth stimulatory effects of angiotensin is available. It is of interest that vascular responses are relatively specific for angiotensin II, with angiotensin III exhibiting potency of 20% or less, whereas adrenocortical responses are equivalent for angiotensin II and III (18, 19, 24). The apparent difference in specificity for angiotensin–receptor interactions may be related to the observed differences in growth response.

Among polypeptide hormones known to stimulate differentiated function of a specific target cell, angiotensin appears unique in that it also stimulates growth *in vitro*. Although it has been reported that follicle-stimulating hormone stimulates DNA synthesis and mitosis of Sertoli cells *in vitro* (29), other polypeptide hormones considered to stimulate growth as well as differentiated function *in vivo* either inhibit cell replication [corticotropin (2, 3, 8, 9)] or have no effect [luteinizing hormone (4–6)] when added directly *in vitro*. Whereas FGF and EGF stimulate cell proliferation in a variety of cell types (1), angiotensin may control organ-specific growth *in vivo* as well as control organ-specific function of the adrenal cortex.

Note Added in Proof. Functional mouse adrenocortical tumor cells (Y-1) did not respond to angiotensin II with either DNA synthesis or steroidogenesis.

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