Induction of Synthesis of Mitochondrial Steroidogenic Enzymes of Bovine Adrenocortical Cells by Analogs of Cyclic AMP*

(Received for publication, July 8, 1983)

Robert E. Kramer‡§, William E. Rainey‡, Bruria Funkenstein‡, Albert Dee‡, Evan R. Simpson‡†, and Michael R. Waterman‡‡

From the Departments of Biochemistry, Cell Biology, and Obstetrics and Gynecology and The Cecil H. and Ida Green Center for Reproductive Biology Sciences, The University of Texas Health Science Center, Dallas, Texas 75235

The long term action of cyclic AMP analogs to stimulate the synthesis of cytochromes P-450sec, P-45011β, and adrenodoxin has been studied utilizing confluent monolayers of adult bovine adrenocortical cells maintained for periods of time up to 72 h in the absence or presence of dibutyryl cyclic AMP (1 mM), 8-bromo cyclic AMP (1 mM), or ACTH (adrenocorticotropic) (10−10 M). The synthesis of these proteins was examined by radiolabeling cellular proteins with [35S]methionine or else by translating RNA extracted from such cells in a cell-free system in the presence of [35S]methionine. In each case, the protein under study was immunoprecipitated utilizing specific antisera, or IgG fractions prepared from such antisera. ACTH and both analogs of cyclic AMP caused an increase in the synthesis of cytochrome P-450sec which reached a maximum 36–48 h after addition, and then declined. On the other hand, butyric acid (1 mM) had no effect on the synthesis of cytochrome P-450sec. Cytochrome P-450sec activity measured as pregnenolone production by both intact cells or isolated mitochondria from such cells was increased following incubation of cells with either dibutyryl cyclic AMP or ACTH. The binding of rabbit anti-cytochrome P-450sec IgG was also increased in cells incubated with dibutyryl cyclic AMP or ACTH as estimated by immunofluorescence microscopy using fluorescein-tagged anti-rabbit IgG. Furthermore, dibutyryl cyclic AMP and ACTH both increased the synthesis of adrenodoxin and of cytochrome P-45011β, as well as the activity of 11β-hydroxylase. Inclusion of ACTH stimulated the secretion of cyclic AMP in a time- and concentration-dependent fashion. Thus, it is concluded that analogs of cyclic AMP can mimic the long term actions of ACTH to induce the synthesis of steroidogenic enzymes, and that this action of ACTH is likely mediated by cyclic AMP.

*This research was supported in part by United States Public Health Service Grants AM28350, HD12331, HD11149, and HD00422 and Grant J-624 from The Robert A. Welch Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†Supported by United States Public Health Service Training Grant 1-T32 HD07190 and the Chilton Foundation. Present address, Department of Pharmacology, University of Tennessee Center for Health Sciences, Memphis, Tennessee 38163.

The actions of ACTH1 to stimulate steroidogenesis in the adrenal cortex can be divided into two categories, short term (acute) actions and long term (chronic) actions. The acute response of the adrenocortical cell to ACTH is believed to involve an increase in the availability of cholesterol to serve as precursor for pregnenolone formation by the mitochondrial cholesterol side chain cleavage system (1–11). This long term action of ACTH is related to the synthesis of specific enzymes involved in steroidogenesis. For example, the levels of various adrenal steroidogenic enzymes including mitochondrial and microsomal forms of cytochrome P-450 decline over a period of several days following hypophysectomy of rats (12–15). On administration of ACTH to such animals the levels of these enzymes increase toward normal values. In other studies, ACTH has been shown to exert long term actions to increase the activity of 11β-hydroxylase (16, 17) and 3β-hydroxysteroid dehydrogenase-isomerase (18) as well as the synthesis of adrenodoxin (19) in mouse Y-1 adrenal tumor cells.

Recently we have utilized bovine adrenocortical cells in primary monolayer culture to study the synthesis of a number of key enzymes involved in steroidogenesis and the regulation of such synthesis by ACTH. Utilizing cells radiolabeled with [35S]methionine we have shown that the synthesis of the mitochondrial enzymes, cytochrome P-450sec (20), cytochrome P-45011β (21), and adrenodoxin (22) is induced in a coordinate fashion, maximal synthesis being obtained after approximately 36 h of exposure to ACTH. Subsequent to this time the rate of synthesis of these enzymes declines in the continuing presence of ACTH, suggestive that the cells become refractory to ACTH. The synthesis of the microsomal enzyme, 21-hydroxylase cytochrome P-450 (steroid 21-hydroxylase), is also induced when the cells are maintained in the presence of ACTH (23), but the time course of induction of this form of cytochrome P-450 differs somewhat from that of the mitochondrial steroidogenic enzymes. Utilizing a rabbit reticulocyte in vitro translation system, and [35S]methionine as the radiolabeled amino acid, we have shown that RNA extracted from cells maintained in the presence of ACTH directs several fold greater synthesis of these various proteins involved in steroidogenesis than does RNA from control cells (20–24). These results suggest that the increase in the synthesis of steroidogenic enzymes in response to ACTH results in part from an increase in transcription of specific mRNA species. Thus, we believe that long term action of ACTH involves the
maintenance of synthesis of specific mitochondrial and microsomal enzymes involved in steroidogenesis.

Ample evidence is available to suggest that the acute action of ACTH is mediated by cyclic AMP (25–27). ACTH treatment has been shown to increase cyclic AMP accumulation by rat and bovine adrenal cells (28–31) as well as by mouse adrenal tumor cells (32–34). Moreover, addition of cyclic AMP analogs such as Bt-cAMP or 8-Br-CAMP can mimic the acute action of ACTH on stimulation of steroidogenesis (28-30, 32). Evidence has been presented that ACTH causes an increase in the amount of cyclic AMP bound to the regulatory unit of cyclic AMP-dependent protein kinase at all concentrations which give rise to an increase in steroidogenesis (35). The role of cyclic AMP as a mediator of the long term effects of ACTH to maintain the synthesis of steroidogenic enzymes has not been studied and thus, it is not known whether this action of ACTH is mediated by cyclic AMP or whether some other second messenger might be involved. Evidence that cyclic AMP mediates induction of synthesis of enzymes by other hormones has been presented. For example, it has been established that synthesis of tyrosine aminotransferase in rat liver is stimulated by cAMP analogs (36). It is also known that the action of glucagon to induce transcription of mRNA species specific for phosphoenolpyruvate carboxykinase in liver can be mimicked by cyclic AMP analogs such as Bt-cAMP (37).

In order to determine whether the action of ACTH to induce the synthesis of mitochondrial steroidogenic enzymes in bovine adrenocortical cells may be mediated by cyclic AMP, we have studied the ability of analogs of this cyclic nucleotide to mimic the inductive effects of ACTH, utilizing bovine adrenocortical cells in monolayer culture as a model system. Our results indicate that cyclic AMP analogs can indeed mimic the effect of ACTH to induce the synthesis of cytochrome P-450, cytochrome P-450, and adrenodoxin.

**EXPERIMENTAL PROCEDURES**

**Cell Culture—**Isolated cells prepared from the zona fasciculata-reticularis of freshly obtained bovine adrenal glands by collagenase digestion and mechanical dispersion were grown in monolayer culture as described previously (20–24). Cells were maintained in culture in 100-mm plastic culture dishes and labware (Oxnard, CA), and collagenase and DNase were from Boehringer (Mannheim, Germany) and obtained from Grand Island Biological Co. (Grand Island, NY). Plastic culture dishes and labware were purchased from Falcon Labware (Oxnard, CA), and collagenase and DNase were from Boehringer Mannheim. ACTH and fibroblast growth factor, fetal calf serum, horse serum, and antibiotics/antimycotics. Upon achievement of confluence, the cells were incubated for an additional 24–48 h in Ham’s F-12/DMEM medium, but in the absence of fibroblast growth factor and antibiotics/antimycotics. At the beginning of an experiment, incubation medium was again replaced with fibroblast growth factor–free and antibiotic/antimycotic-free Ham’s F-12/DMEM medium. Synthetic ACTH, (Cortrosyn, Bt-cAMP, or 8-Br-cAMP were added to the incubation media of the appropriate culture dishes. Thereafter, incubation media were replaced every 24 h. Experiments were continued for 72 h. Cell monolayers were harvested at 12-h intervals to assay enzyme synthesis and activity.

The cortisol contents of the incubation media were measured by direct radioimmunoassay (88) using an antiserum prepared against cortisol-3-carboxymethyloxime-bovine serum albumin. All statistical analyses were by Analysis of Variance, and significantly different means were identified by the method of Least Significant Difference.

**Culture media, sera, and antibiotic/antimycotic solutions were obtained from Grand Island Biological Co. (Grand Island, NY). Plastic culture dishes and labware were purchased from Falcon Labware (Oxnard, CA), and collagenase and DNase were from Boehringer Mannheim. ACTH and fibroblast growth factor, fetal calf serum, horse serum, and antibiotics/antimycotics. Upon achievement of confluence, the cells were incubated for an additional 24–48 h in Ham’s F-12/DMEM medium. Synthetic ACTH, (Cortrosyn, Bt-cAMP, or 8-Br-cAMP were added to the incubation media of the appropriate culture dishes. Thereafter, incubation media were replaced every 24 h. Experiments were continued for 72 h. Cell monolayers were harvested at 12-h intervals to assay enzyme synthesis and activity.

The cortisol contents of the incubation media were measured by direct radioimmunoassay (88) using an antiserum prepared against cortisol-3-carboxymethyloxime-bovine serum albumin. All statistical analyses were by Analysis of Variance, and significantly different means were identified by the method of Least Significant Difference.

**Culture media, sera, and antibiotic/antimycotic solutions were obtained from Grand Island Biological Co. (Grand Island, NY). Plastic culture dishes and labware were purchased from Falcon Labware (Oxnard, CA), and collagenase and DNase were from Boehringer Mannheim. ACTH and fibroblast growth factor, fetal calf serum, horse serum, and antibiotics/antimycotics. Upon achievement of confluence, the cells were incubated for an additional 24–48 h in Ham’s F-12/DMEM medium. Synthetic ACTH, (Cortrosyn, Bt-cAMP, or 8-Br-cAMP were added to the incubation media of the appropriate culture dishes. Thereafter, incubation media were replaced every 24 h. Experiments were continued for 72 h. Cell monolayers were harvested at 12-h intervals to assay enzyme synthesis and activity.

The cortisol contents of the incubation media were measured by direct radioimmunoassay (88) using an antiserum prepared against cortisol-3-carboxymethyloxime-bovine serum albumin. All statistical analyses were by Analysis of Variance, and significantly different means were identified by the method of Least Significant Difference.
Cyclic AMP was determined in the media as described under Experimental Procedures. Incubation, media were collected for determination of extracellular cyclic AMP. An equal volume of perchloric acid (0.6 M) was added to the medium which was allowed to stand for 5 min at 4 °C. After centrifugation at 1000 x g for 15 min at 4 °C, the supernatant was removed and the pH adjusted to pH 6 with KOH (6 N). The samples were incubated for 30 min at 4 °C and centrifuged again at 1000 x g for 30 min. The supernatant was removed, dried, and reconstituted in distilled water. Cyclic AMP was determined by radioimmunoassay using a specific antisera against cyclic AMP which showed a cross-reaction of 4 x 10^-5% with cyclic GMP, 2.6 x 10^-5% with AMP, and 5.9 x 10^-5% with ATP. 2-O-[^32P]Succinyl cyclic AMP tyrosine methyl ester was used as tracer. The assay was performed in acetic acid buffer 0.05 M, pH 6.2. The antigen-antibody complex was precipitated by addition of goat-anti rabbit IgG followed by centrifugation at 1000 x g for 15 min. All reagents for the cyclic AMP radioimmunoassay were obtained from Becton-Dickinson Immunodiagnostics (Orangeburg, New York). The radioactivity in the pellet was determined using a γ-counter.

RESULTS

Formation of Cyclic AMP—Since the purpose of the present study was to investigate whether or not the long-term action of ACTH are mediated by cyclic AMP, it was necessary to establish that ACTH could stimulate cyclic AMP formation by bovine adrenocortical cells prepared and cultured in this laboratory. Confluent cells were incubated with ACTH in various concentrations in the presence of 3-isobutyl-1-methylxanthine, as described under Experimental Procedures. ACTH caused an increase in the accumulation of cyclic AMP in the medium which was both time- and concentration-dependent (Fig. 1). After 15 min of exposure to ACTH, the concentration of ACTH required to cause half-maximal stimulation of cyclic AMP formation was about 5 x 10^-10 M, a value similar to the apparent Kd of ACTH binding to its receptor in rat adrenal cells (40), as well as the concentration of ACTH required for half-maximal stimulation of cyclic AMP formation reported by others (41). The time course of cyclic AMP production in response to various concentrations of ACTH in a separate experiment is shown in Fig. 1B. As indicated in this figure, variability exists in the absolute amount of cyclic AMP produced per unit of time from one cell preparation to another.

Activity of Cytochrome P-450—In order to investigate the effect of Bt2-CAMP on cholesterol side chain cleavage activity, bovine adrenocortical cells were maintained for various periods of time in the absence or presence of Bt2-CAMP (1 mM), ACTH (10^-8 M), or ACTH plus Bt2-CAMP. Medium was then replaced with fresh medium containing SU10603 and trilostane. Incubation was continued for an additional 30 min and pregnenolone in the medium was assayed by radioimmunoassay. Alternatively, after the cells were incubated for various periods of time in the presence or absence of Bt2-CAMP or ACTH, the medium was removed, mitochondria were prepared from cellular homogenates, and pregnenolone formation was assayed as described under Experimental Procedures. The results of these experiments are shown in Fig. 2. Pregnenolone formation by control cells was relatively unchanged throughout the 60-h experiment (Fig. 2A), while exposure of the cells to Bt2-cAMP resulted in a stimulation of pregnenolone formation. Stimulation was maximal at 36 h and approximately 3-fold greater than control values. Thereafter, the rate of pregnenolone formation declined even in the continued presence of Bt2-cAMP. Virtually identical profiles of pregnenolone formation were observed in cells maintained in the presence of ACTH or in the presence of ACTH together with Bt2-cAMP.

When pregnenolone production was assayed in mitochondria prepared from cells incubated in the presence or absence of Bt2-cAMP or ACTH, the pattern of induction was similar to that observed in intact cells (Fig. 2B). Bt2-cAMP caused an increase in activity which reached a maximum by 36 h, and subsequently declined. Similar results were observed using mitochondria prepared from cells incubated in the presence of either ACTH or ACTH plus Bt2-cAMP.

FIG. 1. Effect of ACTH on cyclic AMP secretion by bovine adrenocortical cells in confluent monolayer culture. Cells were incubated with ACTH in various concentrations (A) for 15 min or else for various periods of time (B). At the end of each incubation, cyclic AMP was determined in the media as described under Experimental Procedures.
Synthesis of Cytochrome P-450<sub>acc</sub>—In order to investigate whether or not the increase in cholesterol side chain cleavage activity induced by Bt<sub>2</sub>-cAMP resulted from an increase in the synthesis of cytochrome P-450<sub>acc</sub>, cells were incubated for various periods of time up to 72 h in the presence or absence of Bt<sub>2</sub>-cAMP or ACTH (Fig. 3A) or in the presence or absence of 8-Br-cAMP or ACTH (B). Cell proteins were then radiolabeled with [<sup>35</sup>S]methionine and the synthesis of cytochrome P-450<sub>acc</sub> was determined as described above. In the absence of inducing agents, the incorporation of radiolabel into cytochrome P-450<sub>acc</sub> changed little throughout the course of the experiment. Addition of sodium butyrate (1 mM) had little or no effect on the incorporation of radiolabel into cytochrome P-450<sub>acc</sub> (Fig. 3A). By contrast, incubation of the cells with either Bt<sub>2</sub>-cAMP or 8-Br-cAMP resulted in a marked increase in the incorporation of radiolabel into cytochrome P-450<sub>acc</sub>. A corresponding increase in incorporation was observed in cells maintained in the presence of ACTH. When cells were maintained in the presence of ACTH together with the respective cyclic AMP analog, the effects were not additive. In the experiment presented in Fig. 3B, maximal incorporation of radiolabel was observed at 36 h. Subsequently, the incorporation of radiolabel declined in a manner similar to that previously reported for ACTH (20). In the case of the experiment described in Fig. 3A, the peak of induction did not occur until 48 h. Nevertheless, subsequent to this time, the rate of synthesis of cytochrome P-450<sub>acc</sub> declined even in the continued presence of inducing agents. This shift of 12 h in the development of refractoriness reflects the variability in responsiveness of different preparations of primary cell cultures.

To determine whether or not the action of Bt<sub>2</sub>-cAMP to increase the synthesis of cytochrome P-450<sub>acc</sub> might be due to an increase in the translatability of mRNA species specific for this protein, RNA was extracted from cells incubated in the presence or absence of Bt<sub>2</sub>-cAMP, ACTH, and Bt<sub>2</sub>-cAMP plus ACTH for 36 h. The RNA was translated in vitro in the presence of [<sup>35</sup>S]methionine, and the radiolabeled cytochrome P-450<sub>acc</sub> precursor was immunoprecipitated. Results of such an experiment are shown in Fig. 4. Bt<sub>2</sub>-cAMP increased the in vitro synthesis of cytochrome P-450<sub>acc</sub> approximately 5-fold. ACTH was equally effective in stimulating cytochrome P-450<sub>acc</sub> synthesis, as was ACTH plus Bt<sub>2</sub>-cAMP.

Immunofluorescent Staining of Cytochrome P-450<sub>acc</sub>—Effects of ACTH (1 μM) and Bt<sub>2</sub>-cAMP (1 mM) on the content of cytochrome P-450<sub>acc</sub> within the adrenocortical cell were assayed by immunofluorescence 36 h after addition of inducer. Immunofluorescent staining for cytochrome P-450<sub>acc</sub> was located in the mitochondria (Fig. 5). Although mitochondria were distributed throughout the cell, they were often concentrated in the region of the nucleus. More than 90% of cells maintained in the presence of ACTH (B) or Bt<sub>2</sub>-cAMP (C) demonstrated immunofluorescent staining for cytochrome P-
Induction of Adrenal Hydroxylase Synthesis by Cyclic AMP

450<sub>sec</sub>, that was greater than the control level (A). In contrast, immunofluorescent staining for citrate synthase, a nonsterologenic mitochondrial enzyme, was similar in control cells and cells treated with ACTH or Bt<sub>2</sub>-cAMP. Consistent with these findings, ACTH did not increase the activity of citrate synthase (data not shown).

Synthesis of Adrenodoxin.—To examine whether cAMP analogs were effective to induce the synthesis of another mitochondrial protein involved in the cholesterol side chain cleavage reaction, the ability of Bt<sub>2</sub>-cAMP to induce the synthesis of adrenodoxin was investigated. Cells were maintained for 36 h in the presence or absence of Bt<sub>2</sub>-cAMP, ACTH, or Bt<sub>2</sub>-cAMP plus ACTH. The rate of 11β-hydroxylase activity was increased in the presence of inducing agents (Fig. 7A). When Bt<sub>2</sub>-cAMP was added in the presence of ACTH, it had no additional effect.

Activity of Cytochrome P-450<sub>11α</sub>—11β-Hydroxylase activity was assayed in bovine adrenocortical cells maintained for 36 h in the presence or absence of Bt<sub>2</sub>-cAMP, ACTH, or Bt<sub>2</sub>-cAMP plus ACTH. The rate of 11β-hydroxylase activity was increased in the presence of inducing agents (Fig. 7A). When Bt<sub>2</sub>-cAMP was added in the presence of ACTH, it had no additional effect.

Synthesis of Cytochrome P-450<sub>11α</sub>—The effect of dibutyl cyclic AMP on the synthesis of 11β-hydroxylase was determined in an in vitro translation system directed by total RNA from cells maintained for 36 h in the presence or absence of Bt<sub>2</sub>-cAMP, ACTH, or Bt<sub>2</sub>-cAMP plus ACTH. RNA isolated from Bt<sub>2</sub>-cAMP and ACTH-treated cells directed the synthesis of cytochrome P-450<sub>11α</sub> at approximately three times the control level (Fig. 7B). Nonetheless, the combined effects of Bt<sub>2</sub>-cAMP and ACTH were not greater than that of either inducing agent alone.

FIG. 6. Effects of Bt<sub>2</sub>-cAMP and ACTH on synthesis of adrenodoxin. Cells were incubated for 12-, 36-, or 60 h in the absence or presence of Bt<sub>2</sub>-cAMP or ACTH. The cells were then washed, radiolabeled with [35S]methionine, and the synthesis of adrenodoxin was examined as described under "Experimental Procedures."  , control cells;  , ACTH-treated cells;  , cells treated with Bt<sub>2</sub>-cAMP;  , cells treated with ACTH plus Bt<sub>2</sub>-cAMP for 36 h. The units of synthesis are the same as described in the legend to Fig. 3.
or ACTH for 36 h. At the end of this time, 11β-hydroxylase activity was assayed. Alternatively, RNA was extracted from the cells and translated in vitro in the presence of [³H]methionine, and radiolabeled cytochrome P-450₁₈ precursor was immunoprecipitated as described under “Experimental Procedures.” C, control cells; ACTH, ACTH-treated cells; Bt₂, cells treated with Bt₂-cAMP. The units of synthesis are the same as described in the legend to Fig. 3.

The time course of action of Bt₂-cAMP was very similar to that of ACTH. Moreover, after a maximal rate of synthesis had been achieved, the rate of synthesis then declined even in the continued presence of the nucleotide analog. This onset of refractoriness is similar to that observed in the continued presence of ACTH. Similarly, the actions of another cyclic AMP analog, 8-Br-cAMP, led to induction of the synthesis of cytochrome P-450₁₈. In this case, the increase in synthesis at 36 h was less than that observed with ACTH, although the rate of synthesis did decline at longer times of 8-Br-cAMP treatment. We have observed also a refractoriness of steroidogenic enzymes to continued ACTH stimulation (37).

We conclude from the studies presented here that the action of ACTH to induce coordinately the synthesis of cytochromes P-450₁₈ and P-450₁₉, as well as adrenodoxin (20–22) is mediated via cyclic AMP. The activity of cyclic AMP to induce synthesis of these enzymes is not precisely the same as ACTH with respect to the magnitude of the effect, but the temporal profiles of induction are similar in both cases. Although not proven, the action of ACTH to induce the synthesis of these proteins is likely due to an increase in the synthesis of mRNAs coding for these proteins. In hepatocytes, Bt₂-cAMP has been shown to induce the transcription of mRNA sequences specific for the enzymes tyrosine aminotransferase (36) and phosphoenolpyruvate carboxykinase (37). This is not unreasonable to postulate that cyclic AMP might act in an analogous fashion in the adrenal to initiate the transcription of mRNA sequences specific for steroidogenic enzymes. An important question which remains relates to the identity of the factors which mediate the action of cyclic AMP to initiate transcription of specific mRNA species. It seems likely that cyclic AMP-dependent protein kinase might play an important role in this induction mechanism, but the nature of the specific proteins which are phosphorylated as a result of the activation of protein kinase by cyclic AMP remains to be defined, as does the nature of the steps subsequent to the action of protein kinase which lead to increased synthesis of steroidogenic enzymes.

REFERENCES

1. Brownie, A. C., Simpson, E. R., Jefcoate, C. R., Boyd, G. S., Orme-Johnson, W. H., and Beinert, H. (1972) Biochem. Biophys. Res. Commun. 46, 483–490
2. Brownie, A. C., Alfano, J., Jefcoate, C. R., Orme-Johnson, W. H., Beinert, H., and Simpson, E. R. (1973) Ann. N. Y. Acad. Sci. 212, 344–360
3. Jefcoate, C. R., Simpson, E. R., Boyd, G. S., Brownie, A. C., and Orme-Johnson, W. H. (1973) Ann. N. Y. Acad. Sci. 212, 243–261
4. Alfano, J., Brownie, A. C., Orme-Johnson, W. H., and Beinert, H. (1973) J. Biol. Chem. 248, 789–796
5. Jefcoate, C. R., Simpson, E. R., and Boyd, G. C. (1974) Eur. J. Biochem. 42, 539–551
6. Jefcoate, C. R., and Orme-Johnson, W. H. (1975) J. Biol. Chem. 250, 4671–4677
7. Jefcoate, C. R. (1975) J. Biol. Chem. 250, 4693–4670
8. Williams-Smith, D. L., Simpson, E. R., Barlow, S. M., and Morrison, P. J. (1976) Biochim. Biophys. Acta 449, 72–83
9. Brownie, A. C., Gallant, S., Paul, D. P., Bergon, L. L., Orme-Johnson, N. R., and Orme-Johnson, W. H. (1977) Ann. N. Y. Acad. Sci. 297, 549–560
10. Mason, J. I., Arthur, J. R., and Boyd, G. S. (1978) Mol. Cell. Endocr. 10, 209–233
11. Simpson, E. R., McCarthy, J. L., and Peterson, J. A. (1978) J. Biol. Chem. 253, 3135–3139
12. Doering, C. H., and Clayton, R. B. (1989) Endocrinology 85, 500–511
13. Kimura, T. (1969) Endocrinology 85, 492–499
14. Pfeiffer, D. R., Chu, J. W., Kuo, T. H., Chan, S. W., Kimura, T.,
Induction of Adrenal Hydroxylase Synthesis by Cyclic AMP

and Teben, T. T. (1972) Biochem. Biophys. Res. Commun. 48, 486–490
15. Purvis, J. L., Canick, J. A., Mason, J. I., Estabrook, R. W., and McCarthy, J. L. (1973) Ann. N. Y. Acad. Sci. 212, 319–343
16. Kowal, J. (1969) Biochemistry 8, 1821–1831
17. Kowal, J., Simpson, E. R., and Estabrook, R. W. (1970) J. Biol. Chem. 245, 2438-2443
18. Rybak, S. M., and Ramachandran, J. (1982) Endocrinology 111, 427–433
19. Asano, K., and Harding, B. W. (1976) Endocrinology 99, 977–987
20. DuBois, R. N., Simpson, E. R., Kramer, R. E., and Waterman, M. R. (1981) J. Biol. Chem. 256, 7000–7005
21. Kramer, R. E., Simpson, E. R., and Waterman, M. R. (1983) J. Biol. Chem. 258, 3000–3005
22. Kramer, R. E., Anderson, C. M., Peterson, J. A., Simpson, E. R., and Waterman, M. R. (1982) J. Biol. Chem. 257, 14921–14925
23. Funkenstein, B., McCarthy, J. L., Dus, K. M., Simpson, E. R., and Waterman, M. R. (1983) J. Biol. Chem. 258, 9398–9405
24. DuBois, R. N., Simpson, E. R., Tuckey, J., Lambeth, J. D., and Waterman, M. R. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 1028–1032
25. Schulster, D. (1974) Adv. Steroid Biochem. Pharmacol. 4, 233–256
26. Schulster, D. (1976) in Molecular Endocrinology of the Steroid Hormones (Schulster, D., Burstein, S., and Cooke, B., eds.) pp. 168–207, John Wiley and Sons, New York
27. Gill, G. N. (1976) Pharmacol. Therap. Part B Gen. Syst. Pharmacol. 2, 313–339
28. Rami, S. S., Keri, G., and Ramachandran, J. (1983) Endocrinology 112, 315–320
29. Hayashi, K., Sala, G., Catt, K., and Dufau, M. L. (1979) J. Biol. Chem. 254, 6678–6683
30. Goodyer, C. G., Torday, J. S., Smith, B. T., and Giroud, C. J. P. (1976) Acta Endocrinol. 83, 373–385
31. Duperray, A., and Chambaz, E. M. (1980) Biochem. Pharmacol. 29, 1919–1925
32. Wishnow, R. M., Lifrak, E., and Chen, C. (1976) J. Infect. Dis. 133, 5108–5114
33. Lifrak, E., and Wishnow, R. M. (1978) Biochim. Biophys. Acta 541, 504–514
34. Morera, A. M., Cathiard, A. M., and Saez, J. M. (1978) Biochem. Biophys. Res. Commun. 83, 1553–1560
35. Sala, G. B., Hayashi, K., Catt, K. J., and Dufau, M. L. (1979) J. Biol. Chem. 254, 3861–3865
36. Ernest, M. J., and Feigelson, P. (1978) J. Biol. Chem. 253, 319–322
37. Lamers, W. H., Hanson, R. W., and Meisner, H. M. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 1028–1032
38. Simpson, E. R., Waters, J., and Williams-Smith, D. L. (1975) J. Steroid Biochem. 6, 395–400
39. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
40. Buckley, D. I., and Ramachandran, J. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 7431–7435
41. Moyle, W. R., Kong, Y. C., and Ramachandran, J. (1973) J. Biol. Chem. 248, 2409–2417
42. Kramer, R. E., Simpson, E. R., and Waterman, M. R. (1983) Steroids, in press
43. Benahmed, M., Reventos, J., and Saez, J. M. (1983) Endocrinology 112, 1952–1957
44. Kaukel, E., and Hilz, H. (1972) Biochem. Biophys. Res. Commun. 46, 1011–1018
Induction of synthesis of mitochondrial steroidogenic enzymes of bovine adrenocortical cells by analogs of cyclic AMP.
R E Kramer, W E Rainey, B Funkenstein, A Dee, E R Simpson and M R Waterman
J. Biol. Chem. 1984, 259:707-713.