ACTIVATION OF ADRENAL STEROL ESTER HYDROLASE BY DIBUTYRYL cAMP AND PROTEIN KINASE

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Summary

Direct activation of adrenal Sterol Ester Hydrolase (EC 1.1.1.13) by dibutyryl cAMP, ATP and Mg^{+2} has been demonstrated in adrenal homogenates from three species. Variability in the degree of activation was minimized by preincubation of the tissue homogenate for two hours prior to addition of the cofactors and subsequent enzyme assay. Although baseline sterol ester hydrolytic activity, independent of cofactors, was present in all subcellular fractions, the cAMP-dependent enzyme was primarily associated with the 105,000 x g soluble fraction of the cell. A requirement for protein kinase in the system was demonstrated with a partially-purified enzyme from bovine adrenal.

Organs elaborating steroid hormones, such as adrenal, ovary and testes, are abundant in esterified cholesterol which is localized largely in cytoplasmic lipid droplets (1, 2). The esterified cholesterol appears to function as a reservoir for the free cholesterol required for steroid biosynthesis since administration of a pituitary tropic hormone results in a depletion of the cholesterol esters within the target organ for that hormone (3). In addition, when adrenal steroidogenesis is blocked by administration of cyclohexamide to rats, subsequent administration of ACTH still results in a depletion of the lipid droplets, and accumulation of free cholesterol within the tissue (4). These observations suggest that an ACTH-mediated hydrolysis of sterol esters occurs independent of the hormonal effects on later steps in steroidogenesis.

Behrman and his coworkers (5) have reported that the sterol ester hydrolases in adrenal and corpus luteum are under direct control of specific pituitary hormones, and have a primary regulatory function in steroidogenesis. Although there have been reports of activation of the sterol ester hydrolases of these tissues by cAMP or its derivatives (6-8), experimental data for this

is lacking. The present communication provides evidence for the involvement of cAMP and protein kinase in the activation of the "hormone-sensitive" sterol ester hydrolase in adrenal.

MATERIALS AND METHODS

Rat, dog and bovine adrenal glands were removed immediately after sacrifice of the animals and placed on ice. With dog and bovine adrenals, the medulla was removed and the cortex was scraped from the capsule. This tissue, or whole adrenals from rats, was minced and homogenized in 0.10 M phosphate buffer, pH 7.5, to give a tissue concentration of 15% by weight. Where indicated, the homogenate was incubated for two hours at 37°C to reduce the high baseline sterol ester hydrolase activity, which was probably the result of ACTH-activation of the enzyme during handling of the animals. To one ml of the homogenate was added combinations of ATP (3mM), Mg^{+2} (3mM), dibutyryl cAMP (0.2mM) and protein kinase (32 μg), and the volume was adjusted to 2 ml with phosphate buffer. Following addition of cholesterol-4-^{14}C-oleate (0.2 μc) in 0.1 ml acetone, incubations were carried out at 37°C for two hours. At intervals, 0.1 ml of the incubation medium was added to 1 ml of acetone-ethanol (1:1 v/v). The extent of hydrolysis of the labeled cholesterol oleate was determined as described previously (9). Total protein was determined by the method of Lowry et al (10). Enzyme activity is expressed either as percentage hydrolysis of cholesterol-4-^{14}C-oleate, or as units, where one unit is one umole cholesterol produced per hour at 37°C.

RESULTS AND DISCUSSION

The baseline levels of sterol ester hydrolase in homogenates of the adrenals from the three species (prior to the addition of cofactors) and the effects of addition of db-cAMP, ATP and Mg^{+2} on enzyme activity are summarized in Table 1. Addition of the cofactors together resulted in a marked stimulation in enzyme activity in adrenal homogenates from all three species, but appeared greater with the bovine and rat tissues. In this experiment, and those described below, addition of only ATP and Mg^{+2} had no stimulatory effect on enzyme activity.

TABLE 1 STIMULATION OF ADRENAL STEROL ESTER HYDROLASES BY DIBUTYRYL cAMP, ATP AND Mg^{+2}, AND EFFECT OF PRE-INCUBATION ON REDUCING BASELINE ENZYME ACTIVITY

Tissue or Treatment	% Hydrolysis of Cholesterol Oleate Control	plus cAMP, ATP, Mg^{+2}	% of Control
A. Stimulation of Adrenal Sterol Ester Hydrolase			
Rat Adrenal Homogenate	18.0	29.0	161
Bovine Cortex Homogenate	18.0	34.0	189
Dog Cortex Homogenate	12.0	16.4	137
B. Effect of Pre-Incubation of Bovine Adrenal Cortical Homogenates on Baseline Sterol Ester Hydrolase Activity			
No Pre-Incubation	26.2	25.2	(-4)
2 Hour Pre-Incubation, 37°C	16.1	30.3	188

The incubation medium (2 ml) contained 1 ml of appropriate adrenal homogenate (15% by weight in 0.10 M phosphate buffer, pH 7.5), 3 mM ATP, 3mM Mg^{+2}, 0.2mM dibutyryl cAMP and 0.2 μc cholesterol-4-^{14}C-oleate. Incubations were carried out for 2 hours at 37°C in air. In Table 1-B, Bovine Cortical homogenates were incubated at 37°C for 2 hours prior to addition of substrate and cofactors for enzyme assay (2 hour assay).

Initially, the levels of stimulation of enzyme activity by addition of db-cAMP, ATP and Mg^{+2} were not consistently reproducible since, in many cases, baseline enzymatic activity (absence of cofactors) was already high. However, this activity could be reduced by incubating the adrenal homogenate at 37°C for two hours, or by maintaining the homogenate at 2°C for several days prior to assay of the enzyme. This "preincubation" of the tissue homogenate to reduce baseline sterol ester hydrolase activity is comparable to that used in studies on the hormone-sensitive lipase of adipose tissue (12). The results in Table 1 clearly show a decreased level of enzyme activity due to the incubation of the tissue prior to assay, and also show the ability of added db-cAMP, ATP and Mg^{+2} to reactivate the enzyme.

TABLE 2 SUBCELLULAR DISTRIBUTION OF BOVINE ADRENAL STEROL ESTER HYDROLASE IN THE ABSENCE AND PRESENCE OF DIBUTYRYL cAMP, ATP AND Mg^{+2}

Cell Fraction	Percentage Distribution of Sterol Ester Hydrolase	
	Control	Plus cAMP, ATP, Mg^{+2}
Cell Debris (750 x g Supernatant)	13.3	12.0
Mitochondria (9,000 x g pellet)	23.5	17.1
Microsomes (105,000 x g pellet)	36.6	30.5
Soluble Fraction (105,000 x g Supernatant)	26.5	40.4

The assay media and conditions are described in Table 1 and the text. Assays were for 30 minutes at 37°C. The tissues were not pre-incubated for 2 hours prior to assay.

The possible effect of dilution of the labeled cholesterol oleate in the assay mixture by different levels of endogenous sterol esters of the tissue was not a factor in these studies, since it has been reported elsewhere (2) and confirmed in this laboratory there was no mixing between these two "pools".

The increase in sterol ester hydrolase activity resulting from addition of the cofactors (Table 1) was obtained after 120 minutes of incubation of the assay medium (following 2 hour incubation of the tissue homogenate alone). Samples assayed at 30 and 60 minutes showed a greater difference between the control (no cofactors) and experimental flasks (Table 2). These data show that maximal activation of the enzyme had already occurred by 30 minutes, and that it was not necessary to incubate the enzyme with the cofactors prior to addition of the substrate for enzyme assay, which is in contrast to studies with hormone-sensitive lipase (12).

Data on the cellular localization of bovine adrenal sterol ester hydrolase are summarized in Table 3. As reported earlier (13), all subcellular fractions contained varying levels of hydrolase activity in the absence of

TABLE 3 THE EFFECT OF TIME ON THE ACTIVATION OF STEROL ESTER HYDROLASE OF BOVINE ADRENAL HOMOGENATES

Sampling Time Min.	% Hydrolysis of Cholesterol oleate		% of Control
	Control	plus cAMP, ATP, Mg^{+2}	
30	4.9	17.0	347
60	9.4	22.6	240
120	18.3	39.1	214
180	24.6	49.4	200

Conditions are described in Table 1 and the text.

The tissue homogenate was preincubated prior to enzyme assay.

added cofactors. Addition of db-cAMP, ATP and Mg^{+2} resulted in stimulation of enzyme activity among all cell fractions, with the greatest effect in the 105,000 x g soluble fraction. This is also the major locus of protein kinase in the adrenal (14), which may be, at least in part, responsible for the observed level of enzyme activation in this fraction. The possibility that added protein kinase might further stimulate the enzyme activity observed (with cofactors) in the "mitochondrial" and "microsomal" fractions has not been tested.

Attempts to demonstrate a protein kinase requirement for enzyme activation in crude homogenates of bovine adrenal cortex, or in the 105,000 x g soluble fraction were unsuccessful. Similar negative results had been reported with hormone-sensitive lipase in crude homogenates of adipose tissue (12, 15). The enzyme in the 105,000 x g fraction of bovine adrenal homogenates was purified by ammonium sulfate fractionation (25-40% precipitate redissolved in 10 mM phosphate buffer, pH 7.5) and DEAE-cellulose chromatography. The fraction eluted from the DEAE cellulose column with 10 mm phosphate buffer, pH 7.5, was concentrated by Diaflo ultrafiltration and used for further studies. The data summarized in Table 4 indicate that

TABLE 4 EFFECT OF PROTEIN KINASE ON THE ACTIVATION OF PARTIALLY PURIFIED ADRENAL STEROL ESTER HYDROLASE

Additions[a]	Specific Activity[b]	% of Control
None (control)	5.3	
cAMP, ATP, Mg^{+2}	7.6	131
cAMP, ATP, Mg^{+2} and protein kinase	13.8	221

[a] Conditions are described in Table 1 and the text. Incubations were for 30 minutes at 37° in air.

[b] μmole free cholesterol produced/mg protein/hr.

addition of db-cAMP, ATP and Mg^{+2} to the semipurified enzyme increased the activity of sterol ester hydrolase (131% of control). The further addition of 32 μg of rabbit muscle protein kinase resulted in a further, dramatic increase in enzyme activity.

The data reported in this communication provide direct evidence for activation of adrenal sterol ester hydrolase by cAMP-dependent protein kinase. It remains to be determined whether the mechanism of activation of this "hormone-sensitive" cholesterol esterase is analogous to the enzyme phosphorylation which occurs during activation of the phosphorylase of muscle and liver and the hormone-sensitive lipase of adipose tissue. Irrespective of the mechanism of activation, these studies strongly support the concept (16) that hormonal control of adrenal ester hydrolase should be considered as a primary regulatory site in steroidogenesis by making available the free cholesterol necessary for mitochondrial Δ^5-pregnenolone production.

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