Vol. 89, No. 1, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 12, 1979 Pages 44-49

EFFECT OF ECDYSTERONE ON THE CYCLIC AMP-PROTEIN KINASE SYSTEM IN MOUSE LIVER

R. E. Catalán*, M. D. Aragonés and A. M. Martínez

Departamento de Bioquímica, Colegio Universitario Integrado, Avda. Arcos de Jalón s/n, Madrid-17, Spain

Received May 11,1979

SUMMARY

In vivo administration of ecdysterone produced a decrease in cyclic AMP levels and cyclic AMP-binding protein activity in mouse liver 40 min after injection. These changes were accompanied by a concomitant decrease in cyclic AMP-dependent protein kinase. The effect on phosphoprotein phosphatase was the opposite pattern of that on protein kinase. These results support the idea that the cyclic AMP-protein kinase system may be involved in the heterophylic action of ecdysterone.

The fact that invertebrate hormones may have measurable effects on metabolic and molecular events in vertebrates -hormonal heterophylly- has been recently reviewed (1). In this regard, the heterophylic action of some insect-metamorphosing steroids has been reported and a certain degree of regulation by these steroids on the protein, lipid and carbohydrate metabolisms has been described (2,3,4,5,6).

Analyses of the effects of ecdysterone on high blood glucose levels induced by the administration of glucagon, alloxan or anti-insulin serum, and on the activity of enzymes related to the metabolism of glucose, as well as studies of the incorporation of ^{14}C-glucose into glycogen, have shown a link between the steroid and carbohidrate metabolism (6,7).

Recently, it has been demonstrated that a close relationship exists between ecdysterone and the variations in cyclic AMP and cyclic GMP in mouse serum (8) and further that this relationship can be established between the steroid and the cyclic AMP-protein kinase system in adipose tissue (9).

* Present address: Departamento de Bioquímica, Facultad de Ciencias, Universidad de Madrid, Madrid-3, Spain

0006-291X/79/130044-06$01.00/0

The present study was undertaken to examine the effects of ecdysterone on the cyclic AMP-protein kinase system in mouse liver.

MATERIALS AND METHODS

Experimental conditions for animal treatment and ecdysterone administration have been described previously (8).

Cyclic AMP was measured by a competitive binding protein assay. Cyclic GMP was measured by radioimmunoassay. Extraction, assay conditions and linearization of the data obtained have been described previously (10,11).

Protein kinase activity was measured essentially according to Cherrington et al., (12). The liver homogenate was prepared in a cold buffer (10 mM potassium phosphate, pH 6.8, 10 mM Na_2EDTA, 0.50 mM 1-methyl-3-isobutylxanthine and 150 mM KCl) at a concentration of 1 mg/40 ml. The homogenates were centrifuged at 20,000 x g for 45 min at 4°C, and the supernatant used for the determination of protein kinase activity. The protein kinase assay was performed for 8 min at 30°C in a 30 mM potassium phosphate buffer, pH 6.8, in the presence of 10 mM magnesium acetate, 10 mM sodium fluoride, 0.23 mM γ-^{32}P-ATP, 555 µg of histone, 0.11 mM 1-methyl-3-isobutylxanthine and 50 µl of homogenate + 5 µM cyclic AMP, all in a total volume of 200 µl. The reaction was stopped by adding 0.5 ml of a solution of 10% TCA, and 0.1 ml bovine serum albumin (10 mg/ml). The mixture was filtered through glass filter fiber (Whatman GF/C 83) and the filter was washed five times, each with 5 ml of 10% TCA. The radioactivity of ^{32}P-samples was determined by using a scintillation solution containing 10.5 g PPO, 0.45 g methyl-POPOP, 150 g naphthalene, dioxane to 1500 ml and water to 1800 ml in a Packard liquid scintillation spectrometer model 2425.

Cyclic AMP binding protein activity was measured essentially according to Gilman (13). A 25% liver homogenate was prepared in 0.15 M KCl (w/v). The homogenates were centrifuged at 25,000 x g for 30 min at 4°C and the supernatant used for testing the ^{3}H-cyclic AMP-binding capacity. The assay mixture, in a final volume of 200 µl contained: 50 mM sodium acetate buffer, pH 4.0, 0.2 µM ^{3}H-cyclic AMP and 50 µl of the enzyme preparation. After 90 min of incubation at 0°C, the reaction was

stopped by the addition of 0.5 ml of 20 mM phosphate buffer, pH 6.0, and 0.5 ml of saturated ammonium sulphate. The mixtures were passed through a 0.45 μ Millipore filter. The filters were washed with the buffer and saturated ammonium sulphate and counted as previously indicated for the protein kinase assay.

Phosphoprotein phosphatase activity was measured according to a method previously described (9).

In vitro assays for studying the effects of ecdysterone on phosphatase activity were run at 37°C for 30 min. After incubation, activity was measured as described (9). Purified phosphatase was prepared from mouse liver by means of DEAE chromatography (14).

Protein was determined by the method of Lowry et al. (15).

A statistical analysis of the data was done using Student's t-test (16).

Ecdysterone, calf thymus histone (type IIA) and cyclic AMP-dependent protein kinase from beef heart were obtained from Sigma Chemical Co., St. Louis, USA. All other reagents were of the highest analytical grade available; γ-^{32}P-ATP (sp. act. 5000 Ci/mmol); γ-^{32}P-ATP (sp. act. 16.6 Ci/mmol) and ^{3}H-cyclic AMP (sp. act. 27.5 mCi/mmol) were obtained from The Radiochemical Centre, Amersham, England.

RESULTS AND DISCUSSION

Results are given in Fig. 1. The cyclic nucleotide variation of mouse liver after treatment with ecdysterone is in agreement with previous results described in serum and adipose tissue (8,9). In this regard, the parallel patterns of variation of a cyclic nucleotide in an extracellular fluid such as serum and that of an organ such as the liver support the idea that the metabolic action of ecdysterone in mammals may be mediated in part through the cyclic nucleotide system. Moreover, the variation in protein kinase during the same experiment lends further support to this idea: the similar trends in the variation of the kinase and cyclic AMP levels agree quite well with the established concept that cyclic AMP stimulates the production of a cyclic AMP-dependent protein kinase, thereby inducing protein phosphorylation (17). If the intracellular mechanism of action of cyclic AMP consists of the activation of a protein kinase involved in the phosphoryl-

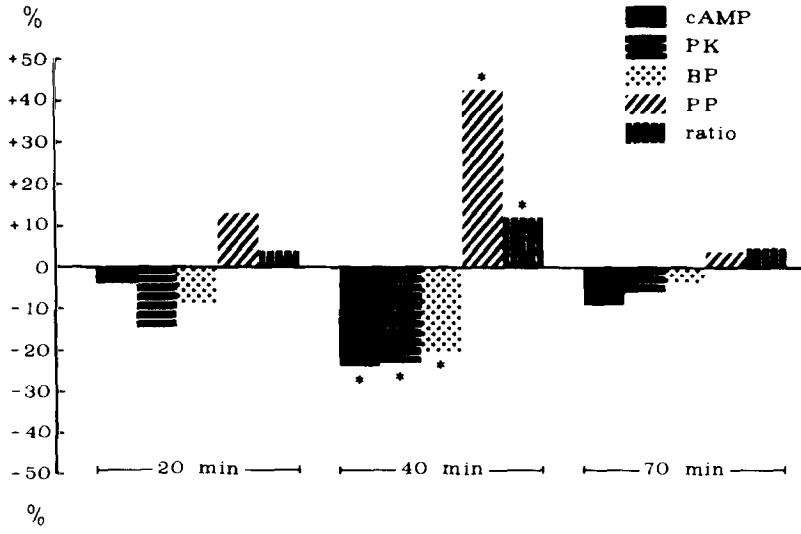

Figure 1. Variations in the cyclic AMP-protein kinase system in mouse liver after administration of ecdysterone. Changes are expressed as percent of the control values (=100%). Asterisks indicate statistically significant changes with respect to the control level.

ation of proteins, the study of phosphoprotein phosphatases (enzymes which reverse the phosphorylating reaction) could be useful in deducing the mechanism by which protein phosphorylation regulates cellular function. In this regard, the effect produced by ecdysterone treatment on phosphoprotein phosphatase, opposite that found for protein kinase, should be interpreted as further support of the results obtained with respect to the variations in cyclic AMP levels.

Some relationship between the insect-metamorphosing hormones and the cyclic nucleotide system in certain species of insects has been established (18,19); nevertheless, results contradicting such findings have also been reported (20,21). Although the suggestion that cyclic AMP could be the second messenger for some steroid hormones has not gained general support (22) sight should not be lost of the fact that the in vivo administration of steroid hormones produces variations in the endogenous phosphorylatipn of individual proteins in rat tissues (23); these proteins affected by cyclic AMP action can be regulated either by steroids in its production or affecting its ability to become phosphorylated. The protein known as

Table 1

In vitro effect of ecdysterone on phosphoprotein phosphatase activity

Additions	Phosphatase activity*
None	706
Ecdysterone 10^{-6} M	1192
" 10^{-5} M	1259
" 10^{-4} M	1581

*Values are given in arbitrary units (cpm/50 µl)

SCARP (steroid and cyclic AMP regulated phosphoprotein) could represent a factor which plays a role in the mechanism through which the steroids achieve some of their biological effects. As it has been reported that steroid hormones could induce the synthesis of a phosphoprotein phosphatase intervening in the dephosphorylation process, the in vitro effect of ecdysterone on a partially purified phosphoprotein phosphatase from mouse liver was measured in an attempt to better understand the mechanism of action of the hormone. The preliminary results given in Table 1 show that ecdysterone stimulates phosphatase activity as was found in the in vivo experiments described above.

Current studies are under way to determine the effect of ecdysterone on the catalytic subunit, regulatory subunit and modulator protein.

Acknowledgements

The authors wish to express their gratitude to Ms. S. Periáñez, Mr. J. E. Godoy, Ms. B. G. Miguel, Mr. A. Robles and Ms. R. G. Hoyo for their valuable technical assistance.

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