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EFFECT OF ECDYSTERONE ON CYCLIC AMP AND CYCLIC GMP IN MOUSE PLASMA

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SUMMARY

Cyclic nucleotide levels were determined in mouse plasma at several times after the administration of ecdysterone. Cyclic AMP exhibited a significant decrease after 40 minutes; an inverse pattern of variation was exhibited by cyclic GMP. These results suggest that the heterophylic action of ecdysterone in mammals could be mediated through the cyclic AMP system. These results could also support the relationship between the ecdysterone and the cyclic AMP-protein kinase system and the synchronous variations undergone by cyclic AMP and cyclic GMP described in several biological systems.

The concept of hormonal heterophylly that invertebrate hormones have some effects on metabolic and molecular events on vertebrates, has been recently reviewed (1). The heterophylic action of some insect-metamorphosing steroids has been described, indicating that a certain degree of regulation by this steroid on protein, lipid and carbohydrate metabolism takes place.

Ecdysone-like steroids obtained from plants can stimulate the incorporation of amino acid into protein in mouse liver (2) a fact that has been explained in terms of its effect on the ability of ribosome to assemble amino acids into polypeptide chains (3); it has also been indicated that ecdysterone acts on protein synthesis by regulating the synthesis of messenger and other types of RNA (4).

Effects of ecdysterone on lipid metabolism have also been described. Experimental atherosclerosis in rabbits indicated

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that the increased levels of total and free cholesterol in serum induced by cholesterol feeding, were significantly inhibited by oral administration of the hormone (5). Incorporation of ^{14}C-acetate *in vivo* into cholesterol in liver was decreased after treatment with ecdysterone, but the rate of disappearance of ^{14}C-cholesterol from the blood was apparently unchanged by treatment with ecdysterone (6).

Studies on 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 involved in the metabolism of glucose and incorporation of ^{14}C-glucose into glycogen have shown a close relationship between the hormone and carbohydrate metabolism (6,7).

On the other hand, cyclic AMP has been suggested to be the second messenger which mediates the action of a variety of hormones in different animal tissues (8). Since the initial demonstration of Sutherland et al. (9) that epinephrine stimulates the adenyl cyclase system of liver cells, numerous other hormones, including steroid hormones, have been shown to influence this enzyme (10-12). Other reports have indicated some relationship between the insect-metamorphosing hormones and the cyclic nucleotide system in certain species of insects (13-15).

It is the aim of the present work to examine the effects of ecdysterone on cAMP and cGMP levels in mouse plasma.

MATERIALS AND METHODS

Male mice weighing 18-20 g were used throughout these experiments. Animals were maintained on a standard laboratory diet with water ad libitum. Animals were starved for one night prior to injection. Ecdysterone was dissolved in 0.9% saline and injected intraperitoneally in a dose of 10 µg per mouse. Controls were handled in the same way except saline was injected instead of ecdysterone.

The mice were killed by decapitation and blood was collected to 1% of its volume of 0.5 M EDTA, pH 7.5 contained in a cooled centrifuge tube. Cyclic AMP was measured by a competitive binding protein assay. Cyclic GMP was measured by a radioimmunoassay. Assay conditions and linearization of data obtained have been described previously (16,17).

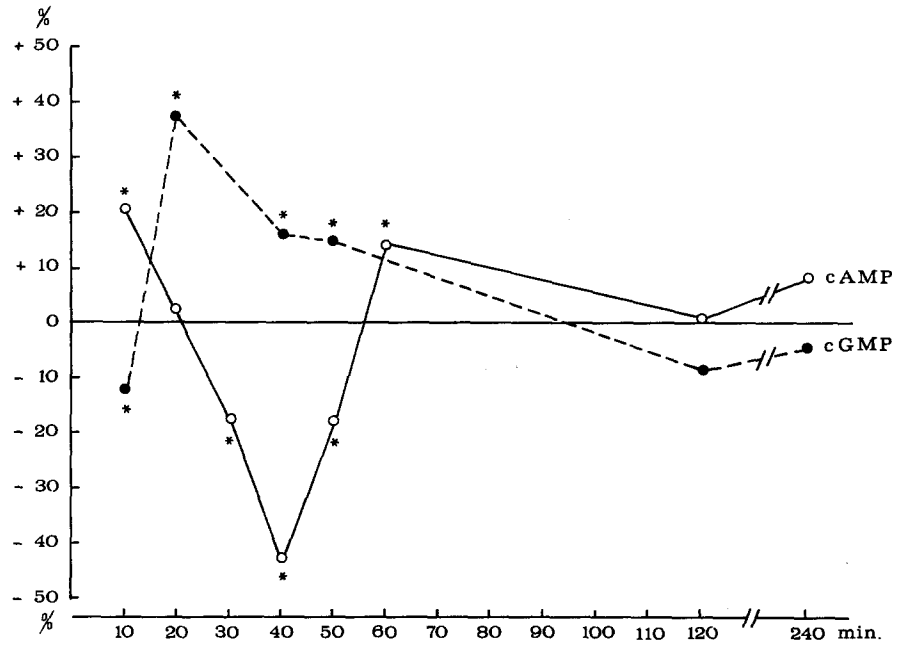

Figure 1.- Variations of cyclic nucleotides in mouse plasma after ecdysterone administration. The changes in nucleotide levels are expressed as percent of the control values (=100%). Asterisks indicate statistically significant changes from the control level.

Protein was determined by the method of Lowry et al. (18).

Statistical analysis of the data was done using Student's t-test (19).

Ecdysterone was obtained from Sigma Chemical Co., St. Louis. U.S.A. All other reagents were of the highest analytical grade available.

RESULTS AND DISCUSSION

Figure 1 shows the effect of ecdysterone on nucleotide levels in mouse plasma obtained in the time-course experiments. An increase in cyclic AMP during the first minutes was observed, a fact that could be due to the stress on the mice at the time of injection, since fluctuations were also observed in the control animals.

Levels of cyclic AMP showed a sharp decrease after 40 min. These changes coincide with opposite variations in cyclic GMP levels.

With regard to the cyclic AMP level variation it should be noted that ecdysterone has been recognized to have a suppresive effect on hyperglycemia induced by several compounds, including glucagon, after 30 min in time course experiments. The fact that such a modification could take place only when ecdysterone was given prior to the administration of glucagon was interpreted to be an effect not produced by direct action of the steroid on glucagon action, but rather coupled with some changes in the amount of a certain mediator or in a metabolic system. The mechanism(s) involved in this action are unknown (7). Since there are data indicating a close relationship between the cyclic nucleotide system and glucagon action (20-23), these results allow us to assume that variations in glucagon actions due to ecdysterone treatment mentioned above may be mediated by cyclic AMP levels.

Considerable evidence has been accumulated in support of the "Ying-Yang" hypothesis (24) that a reciprocal dualism exists between the two nucleotides, so that the ratio of the two compounds within cells might thus regulate many cellular functions. The two nucleotides could act cooperatively in a monodirectional system as positive effectors of different sequential steps or as intracellular mediators of different stimulatory extracellular signals, which in varying combinations could produce somewhat different qualitative or quantitative responses. Although the role played by cyclic GMP on ecdysterone action remains to be determined, the data from these studies suggest that different nucleotide concentrations may represent active and passive signals for the hormone.

Nevertheless, we must point out that the mechanism by which the ecdysterone acts, and its relationship with the cyclic AMP system is difficult to postulate, because the suggestion that some steroid hormones may use cyclic nucleotides as second messengers has not gained general support (25) and even in insects some contradictory results between the ecdysterone and adenyl cyclase have been described (26,27). On the other hand it is possible that ecdysterone could present a more defined action compared to mammal steroids so far studied, or be able to act on other systems related to cyclic nucleotides. One can imagine that this heterophylic phenomenon could be a suitable model to gain insight into the problem cited above.

Clearly the analysis of the relationship between the ecdysterone and the cyclic AMP system deserves a more detailed study.

In conclusion, our results suggest that some heterophylic actions of ecdysterone in mammals are probably mediated through the cyclic AMP system. At the same time this data gives additional support to the relationship between ecdysterone and the cyclic AMP-protein kinase system described in invertebrates and also to the synchronous variation undergone by cyclic AMP and cyclic GMP described in several biological systems.

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