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EFFECT OF ECDYSTERONE ON HYPERGLYCEMIA IN EXPERIMENTAL ANIMALS

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Abstract—Ecdysterone, one of the insect-metamorphosing steroids isolated from plants (Amaranthaceae: *Achyranthes fauriei*), has been recognized to have a suppressive effect on hyperglycemia induced by several hyperglycemic agents. While the administration of ecdysterone did not alter the blood glucose level of normal animals, pretreatment with ecdysterone prior to hyperglycemic agents suppressed the hyperglycemia induced both by glucagon and by anti-insulin serum at only low levels as well. The effect of ecdysterone was also demonstrated using alloxan-diabetic animals. After the administration of ecdysterone to alloxan-diabetic mice, the blood glucose level was reduced to about one-half of the value observed before the administration of ecdysterone. The incorporation of [^{14}C]glucose into protein of normal mouse liver and into glycogen of normal and mildly diabetic mouse liver was stimulated by treatment with ecdysterone.

IT HAS been reported that ecdysterone (Fig. 1) and many other insect-metamorphosing steroids from plant kingdoms show high protein anabolic activity in mouse liver.[1] Since the potent anabolic steroid, 17-ethyl-19-nortestosterone, and other nortesto-

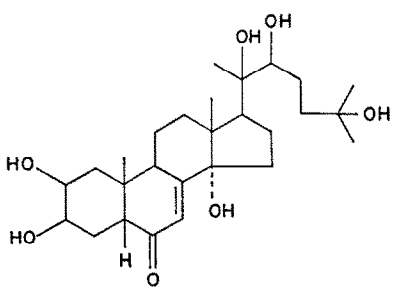

FIG. 1. The structure of ecdysterone: 2,3,14,20,22,25-hexahydroxy-5β-cholest-7-ene-6-one.

sterones are effective in reducing the hyperglycemic response due to exogenous glucagon in human subjects and in laboratory animals,[2–4] suggesting that anabolic steroids may regulate carbohydrate metabolism, it became of interest to know whether ecdysterone can also influence carbohydrate metabolism.

This paper deals with the effect of ecdysterone on high blood glucose levels induced by the administration of glucagon, alloxan or anti-insulin serum (AIS) in rats and mice.

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MATERIALS AND METHODS

Animals and treatment. Male Donryu rats weighing 200 g and male ddy mice weighing 20 g and maintained on a standard laboratory diet were used. Glucagon was injected intraperitoneally (0·2 mg/kg of body weight) into animals that were starved for 6 or 3 hr prior to the injection. Alloxan diabetes was induced by a single intravenous injection of alloxan monohydrate (70 mg/kg of body weight) into animals starved for 24 hr. At 5–7 days after the injection, animals were selected and used for experiments, after confirmation of high blood glucose levels. AIS (100 or 350 mg/kg of body weight) was injected intravenously into mice. Ecdysterone was injected intraperitoneally by the various techniques shown in each experimental set-up.

Analyses. Blood sampling was performed by orbital puncture and cardiac puncture in the mouse and rat respectively. Blood glucose levels were determined by glucose oxidase[5] and by 3,6-dinitrophthalic acid[6] in the rat and mouse respectively. [^{14}C]-glucose incorporation into the hot acid-insoluble fraction was assayed as described previously.[1] [^{14}C]glucose incorporation into glycogen was determined according to Hassid and Abraham.[7]

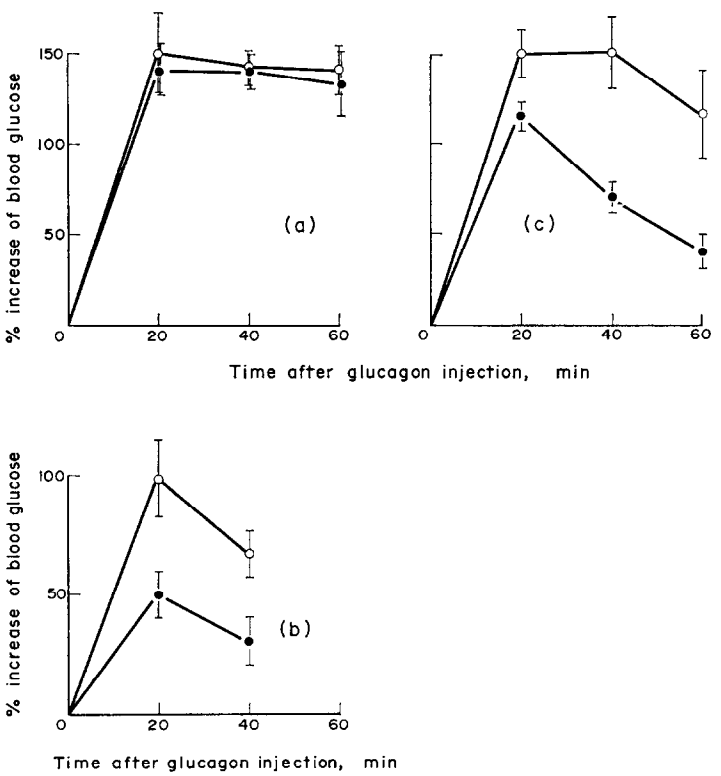

FIG. 2. Effect of a single dose of ecdysterone on hyperglycemia induced with glucagon. Ecdysterone (0·5 mg/kg) was given in the following ways: (a) simultaneously with glucagon; (b) 1 hr before glucagon injection; and (c) 2 hr before glucagon injection. Average initial blood glucose levels were 83 ± 6 mg/100 ml in a, 90 ± 7 mg/100 ml in b, and 77 ± 3 mg/100 ml in c. The vertical bars are standard errors of the mean of 10 mice. Control, ○——○; ecdysterone, ●——●.

Chemicals. Uniformly labeled D-[^{14}C]glucose (specific activity, 4·8 mc/m-mole) was purchased from Daiichi Chemical Company. Glucagon was obtained from Eli Lilly & Company, and alloxan monohydrate from Wako Pure Chemical Industries, Ltd. AIS was harvested from guinea pigs that were treated with repeated injections of crystalline bovine insulin with BCG as adjuvant.[8] The serum that was obtained was lyophilized. One mg of AIS neutralized the activity of 2 mU of bovine insulin. Ecdysterone was isolated and crystallized as reported previously.[9]

RESULTS

Effect of ecdysterone on hyperglycemia evoked by glucagon. Figure 2 shows the effect of ecdysterone on glucagon-induced hyperglycemia in mice. The single administration of glucagon elevated blood glucose levels. When ecdysterone was injected simultaneously with glucagon, suppressive effect was not observed. The elevation of blood glucose tended to be suppressed by injection of ecdysterone 1 or 2 hr prior to the administration of glucagon. Ecdysterone also shortened the period required for the restoration of blood glucose levels to normal, and depressed the maximum content of glucose in blood induced by glucagon. Such modification of blood glucose was not demonstrated in normal animals either in the fasted or nonfasted states.

Effect of ecdysterone on hyperglycemia induced by alloxan. After the injection of ecdysterone, as shown in Fig. 3, mice recovered from alloxan-induced hyper-

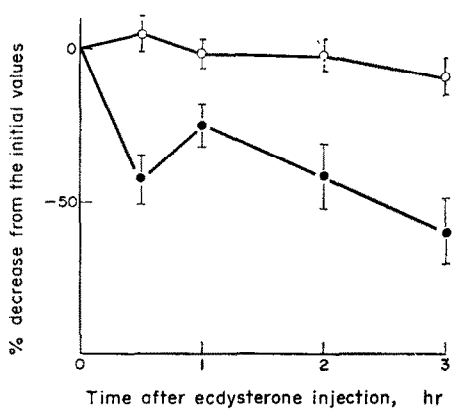

FIG. 3. Effect of a single dose of ecdysterone on hyperglycemia induced with alloxan in mice. Mice were used 5–7 days after alloxan injection. Average initial blood glucose levels were 245 ± 15 mg/100 ml. The vertical bars indicate standard errors of the mean of 10 mice. Control, ○——○; ecdysterone, ●——●.

glycemia. At about 3 hr after the administration of ecdysterone, the glucose level was reduced about 60 per cent from the initial hyperglycemic value. In alloxan-diabetic rats (blood glucose <400 mg/100 ml), a significant decrease of blood glucose was observed within 2 hr after the administration of ecdysterone. However, rats with severe alloxan diabetes (blood glucose >400 mg/100 ml) were not responsive to ecdysterone (Table 1).

Effect of ecdysterone on hyperglycemia caused by AIS. At about 90 min after AIS injection, the blood glucose of mice reached maximum. When ecdysterone was injected 1–3 hr before AIS injection (100 mg/kg), the hyperglycemic response to

TABLE 1. EFFECT OF A SINGLE DOSE OF ECDYSTERONE ON HYPERGLYCEMIA INDUCED WITH ALLOXAN IN RATS*

Treatment (mg/kg)	Within 400 mg/100 ml			More than 400 mg/100 ml		
	Before	After	% Change	Before	After	% Change
Ecdysterone (0·1)	316 ± 14	278 ± 16	−12·1 ± 1·3(5)	558 ± 27	569 ± 38	+2·1(3)
Ecdysterone (0·5)	307 ± 27	249 ± 24	−18·9 ± 3·2(6)	547 ± 44	565 ± 45	+3·4(3)
Ecdysterone (1)	344 ± 16	280 ± 17	−18·4 ± 3·4(5)	586 ± 46	588 ± 16	+4·5(3)
Ecdysterone (10)	362 ± 14	298 ± 14	−17·7 ± 2·4(5)	552 ± 45	561 ± 44	+1·9(5)

* Rats were used 7 days after the administration of alloxan, and received an injection of ecdysterone 2 hr before death. Values shown are means ± S.E. Figures in parentheses indicate the number of observations in each group.

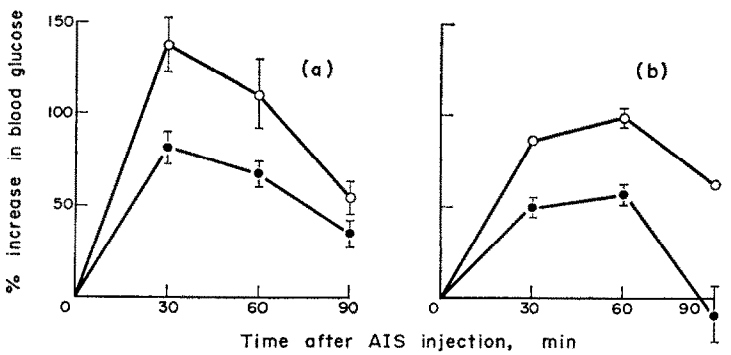

FIG. 4. Effect of a single dose of ecdysterone on hyperglycemia induced with AIS in mice. Ecdysterone (0·5 mg/kg) was administered in the following ways: (a) 1 hr before AIS (100 mg/kg) injection, and (b) 3 hr before AIS (100 mg/kg) injection. Average initial blood glucose levels were 78 ± 4 mg/100 ml in a, and 76 ± 5 mg/100 ml in b. The vertical bars indicate standard errors of the mean of 10 mice. Control, ○——○; ecdysterone, ●——●.

AIS was suppressed, while with the hyperglycemia caused by injection of higher doses of AIS (350 mg/kg), the effectiveness of ecdysterone tended to diminish (Figs. 4 and 5).

Incorporation of [^{14}C]*glucose into protein and glycogen of mouse liver.* The ability of ecdysterone to stimulate glucose incorporation into protein and glycogen fractions of mouse liver was employed as one of the indexes to estimate glucose utilization *in vivo*. As shown in Table 2, stimulation of the incorporation of glucose into protein

TABLE 2. EFFECT OF ECDYSTERONE ON [^{14}C]GLUCOSE INCORPORATION INTO PROTEIN OF MOUSE LIVER*

Treatment	Counts/min/mg Protein	Stimulation (%)
Control	120 ± 2	100
Ecdysterone, 2 hr	160 ± 7	133
Ecdysterone, 4 hr	180 ± 3	150

* Normal mice were used. Mice were killed 1 hr after the injection of [^{14}C]glucose (2 μc/0·1 ml). The results are the means ± S.E. of 6 mice per group.

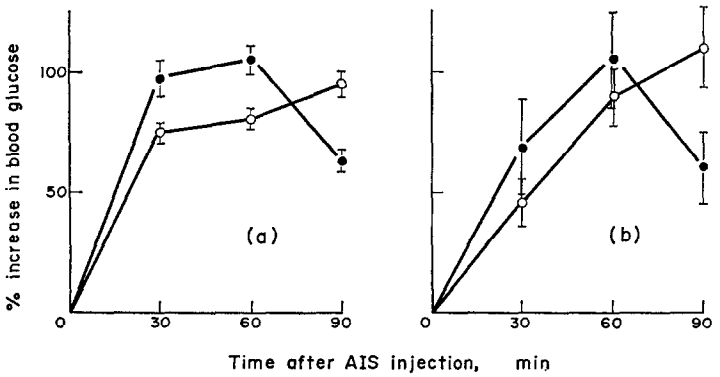

FIG. 5. Effect of a single dose of ecdysterone on hyperglycemia induced with a higher dose of AIS in mice. Ecdysterone (0·5 mg/kg) was administered in the following ways: (a) 1 hr before AIS (350 mg/kg) injection, and (b) 3 hr before AIS (350 mg/kg) injection. Average initial blood glucose levels were 78 ± 5 mg/100 ml in a, and 73 ± 3 mg/100 ml in b. The vertical bars indicate standard errors of the mean of 8 mice. Control, ○——○; ecdysterone, ●——●.

was observed at 2 and 4 hr after the administration of ecdysterone, a stimulation of the same magnitude as the effect of the steroid on the incorporation of amino acids into protein, which was reported previously.[1] The incorporation of [^{14}C]glucose into glycogen was also stimulated by administration of ecdysterone (shown in Table 3).

TABLE 3. EFFECT OF ECDYSTERONE ON [^{14}C]GLUCOSE INCORPORATION INTO GLYCOGEN OF MOUSE LIVER*

Treatment	Counts/min/g wet wt. liver
Normal mice	
Control	3660 ± 380
Ecdysterone, 1 hr	5750 ± 820
Alloxan-diabetic mice	
Experiment 1	
Control	1230 ± 120
Ecdysterone, 1 hr	2060 ± 180
Experiment 2	
Control	660 ± 50
Ecdysterone, 3 hr	1140 ± 170

* Normal and mildly diabetic (blood glucose, 220–280 mg/100 ml) mice were used. Mice were killed 20 min after the injection of [^{14}C]glucose (2 μc/0·1 ml). The results are the means ± S.E. of 6 mice per group.

DISCUSSION

It has been shown that certain nortestosterone compounds depress the normal hyperglycemic response to glucagon in human subjects and in laboratory animals.[2–4] In this paper, a depressive effect of ecdysterone on hyperglycemia induced by several hyperglycemic agents was demonstrated. Ecdysterone had no effect on the normal

blood glucose level. However, ecdysterone apparently reversed the hyperglycemia induced by glucagon. The fact that such modification can take place only when ecdysterone is given prior to the administration of glucagon indicates that this effect is not produced by direct action of ecdysterone on glucagon, but is coupled with some changes in the level of a certain mediator or metabolic system. Ecdysterone was also found to be effective in modifying the hyperglycemic response to AIS. In this case, however, ecdysterone did not reduce the hyperglycemic response caused by an overdosage of AIS. In alloxan-diabetic mice, a remarkable decrease of blood glucose induced by the administration of ecdysterone was observed. The effect was considerably less in rats. The mechanism(s) involved in this action of ecdysterone is unknown.

The hyperglycemic response to glucagon is usually interpreted as an index of hepatic glycogenolysis; however, it has also been suggested that the gluconeogenic action of glucagon is most likely responsible for the hyperglycemia.[10] Hazelwood and O'Brien[2] suggested that norethandrolone acts by blocking gluconeogenesis, leading to diminished stores of liver glycogen. AIS also stimulates gluconeogenesis.[11,12] Since the actions of glucagon and AIS on gluconeogenesis may be similar, it is conceivable that ecdysterone depresses the hyperglycemia by preventing the augmented gluconeogenesis in a manner similar to that of norethandrolone.

The incorporation of [^{14}C]glucose into the hot acid-insoluble fraction, namely protein, was stimulated by treatment with ecdysterone for 2 or 4 hr, as well as the incorporation of ^{14}C-glucose into glycogen. Thus, elevated glucose utilization may be one of the mechanisms involved. The fact that ecdysterone could not modify the hyperglycemia induced by the higher dose of AIS and could not reduce the hyperglycemia in severe alloxan-diabetic rats suggests that ecdysterone may require the availability of a certain amount of insulin or at least minimized function of the pancreas.

Although many possibilities are available to explain the observed results, further study will be needed to ascertain the detailed mechanism involved.

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