EFFECT OF HYDROCORTISONE ON ALKALINE PROTEINASE ACTIVITY IN RAT TARGET ORGANS

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Glucocorticoids and their synthetic analogs inhibit protein metabolism in the thymus [5, 8]. The thymolytic action of these compounds, which is observed both in vivo and in vitro, depends on the dose of the hormone and causes death of the thymocytes [9]. Meanwhile, glucocorticoids stimulate anabolism in the liver cells, and intensify total protein synthesis [1]. A catabolic effect of glucocorticoids is observed in rat skeletal muscles, and in recent years alkaline phosphatases (AlP), induced by these hormones, and nonlysosomal enzymes with pH optimum in the alkaline region, have been found in these muscles [6, 7, 10, 13].

The aim of this investigation was to determine the presence of AlP in the thymus and liver of rats and also to discover their sensitivity to the action of glucocorticoids.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 200-220 g. Hydrocortisone (HC; from "Serva," Germany) was injected into the experimental animals and the doses and duration of action of the hormone are indicated below. The conditions of injection of the hormone and determination of AlP activity in homogenates of the thymus and liver were described previously [3]. The protein concentration was determined by Lowry's method [12]. To study the pH-dependence of proteolytic activity of the homogenates Tris-HCl buffer, pH 7.0-9.5 was used. Confidence intervals of the experimental values were calculated and the significance of differences between them determined by Student's test at a level of significance of $p < 0.05$.

EXPERIMENTAL RESULTS

Comparison of the pH-dependence of proteolytic activity of homogenates of the thymus of intact and experimental rats showed that 24 h after injection of HC in a dose of 15 mg/kg a peak of proteinase activity appeared in the region of pH 8.5-9.0 (Fig. 1). In the liver of the experimental animals, at the same pH values, a decrease in proteolysis of the substrate was recorded.

The experimental results obtained during the study of AlP activity of the thymus and liver of the rats 24 h after a single injection of HC in doses of 1, 5, 15, and 25 mg/kg are given in Fig. 2. In all doses studied the hormone activated AlP in the thymus, and the enzyme was most strongly induced by HC in doses of 15 and 25 mg/kg, at which a significant difference was observed between activity in the experimental and control series. In the liver, HC in all doses reduced AlP activity, although no significant differences were found in the degree of inhibition of the enzyme by the hormone.

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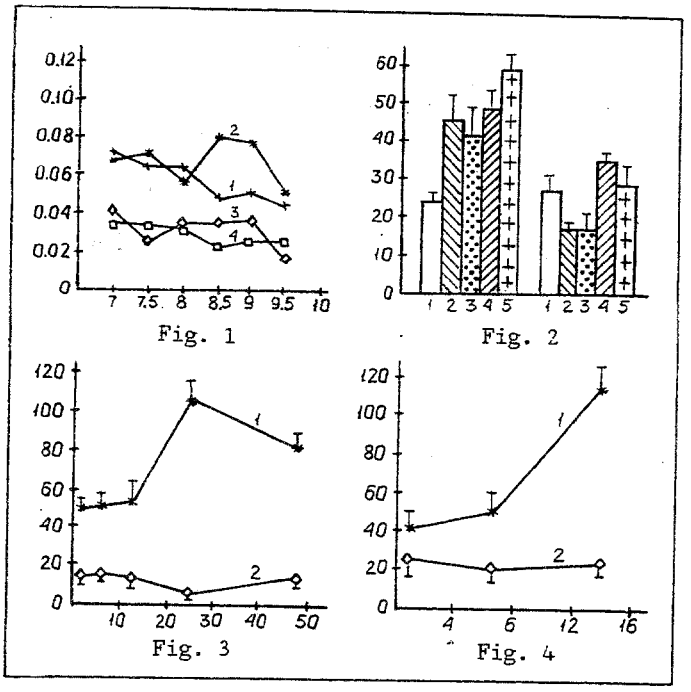

Fig. 1. Proteolytic activity of homogenates of thymus and liver of intact (1, 3) and HC-induced (2, 4) rats in region of neutral and alkaline pH values. Abscissa, pH; ordinate, specific activity (in U/mg protein/h).

Fig. 2. Effect of a single injection of HC on alkaline proteinase activity of rat thymus and liver homogenates. Ordinate, specific activity (in U/mg protein/h): 1) enzyme activity in control animals, 2) enzyme activity 1 day after injection of HC in a dose of 1 mg/kg, 3) HC in a dose of 5 mg/kg, 4) HC in a dose of 15 mg/kg, 5) HC in a dose of 25 mg/kg.

Fig. 3. Change in activity of proteinases in homogenates of rat thymus (1) and liver (2) after a single injection of HC in a dose of 15 mg/kg. Abscissa, duration of action of HC (in h); ordinate, specific activity (in U/mg protein/h).

Fig. 4. Changes in proteinase activity of rat thymus (1) and liver (2) homogenates in response to chronic injection of HC in a dose of 5 mg/kg. Abscissa, duration of injection of hormone (days); ordinate, specific activity (in U/mg protein/h).

The study of the time course of AlP activation in the thymus of the intact rats after a single injection of HC (15 mg/kg) showed that a steady increase in proteolytic activity was recorded after 6 h, reaching a maximum after 24 h (Fig. 3). After 48 h a decrease in the degree of activation of AlP was observed, although compared with the basal level, activity of the enzyme remained increased. In the liver the maximal decrease in AlP activity was observed 24 h after injection of the hormone.

The results are in agreement with the generally accepted model of the action of steroid hormones, according to which the biological response of an effector cell to a hormonal stimulus is mediated by depression of certain regions of the genome followed by synthesis of specific proteins [2, 4, 11]. The latent period is about 4 h and the maximal response of the cell develops toward 12-24 h after injection of the hormone [1].

Injection of HC into intact rats in a dose of 5 mg/kg during 1 day increased the proteinase activity of the thymus for 7 days (Fig. 4). By the 14th day of daily administration of the hormone in this dose AlP activity increased to more than twice the control level.

The following conclusions can accordingly be drawn: 1) proteolytic enzymes sensitive to the action of HC, active in the alkaline pH region, are present in the liver and thymus of rats; 2) the character of the change in activity of these enzymes during hormone administration differs: AlP activity in the thymus is enhanced by HC, whereas in the liver, it is inhibited; 3) activation of AlP in the thymus depends on the dose, time of action, and plan of administration of the hormone.

It can be tentatively suggested that the difference in the character of action of HC on metabolism in the thymus and liver can be explained by a change in activity of AlP of competent cells relative to the basal level.

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