MECHANISM OF HORMONE ACTION
The effect of synthetic corticosteroids on liver RNA, and hepatic ribonuclease and ribonuclease inhibitor activities in rats

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1. Introduction

The gluconeogenic effect of corticosteroids is believed to be caused by marked increases in the activities of enzymes unique to the process and is possibly caused by *de novo* synthesis of these enzymes [1–3]. The synthesis of enzyme protein, in fact all proteins, depends among many factors on the stability of polysomes and adequate supply of amino acids [4]. The stability of polysomes is, on the other hand, directly related to the stability of RNA, which is directly controlled by ribonuclease activity and indirectly by ribonuclease inhibitor activity [5–7].

Although in mammalian cells stabilities of polysomes and RNA appear to be at least partly controlled by the activities of ribonuclease and its inhibitor very few studies relating to the effects of corticosteroids on them have been made, compared to the extensive investigations carried out on the effects of these steroids on RNA, protein and carbohydrate synthesis. The results of the study relating to the changes in glucose production, liver RNA, and the activities of ribonucleases, ribonuclease inhibitor and hepatic aspartate and alanine aminotransferases in rats after the administration of triamcinolone diacetate, are described in this paper.

2. Materials and methods

Male rats weighing from 110 to 120 g were purchased from Canadian Breeding Company, St.Constance la Prairie, Quebec and maintained on a standard laboratory diet (rat chow). The administration of triamcinolone diacetate was the same as previously described [8,9]. The preparation of cell-free extract (post-nuclear fraction), mitochondria and high-speed supernatant fraction from a 10% liver homogenate was carried out according to the method described by Schnider and Hogeboom [10]. The first two preparations were subjected to repeated (4 times) freezing and thawing prior to use as the source of ribonuclease activities [11] which were measured by the method of Kunitz [12]. Ribonuclease inhibitor activity was measured in the high-speed supernatant fraction at pH 8.0 by the method of Rahman [13], using crystalline bovine pancreatic ribonuclease (Worthington) as the enzyme and highly polymerized yeast RNA (Calbiochem), dialized against 0.002 M EDTA, as the substrate. The activities of aspartate and alanine aminotransferases and blood glucose concentration were determined by the methods described in previous papers [8,14], and the total RNA by the method of Fleck and Begg [15].

3. Results

A sharp increase in glucose production followed by a rapid drop was observed in rats (fig. 1) after hormone administration. Maximum glucose production was noticed from 60 to 70 hr when 2.5 mg of hormone per 100 g of body weight was administered, from 85 to 95 hr when 5 mg per 100 g of body weight was administered, and the values attained were 164 mg, 190 mg, and 224 mg respectively. The blood glucose levels

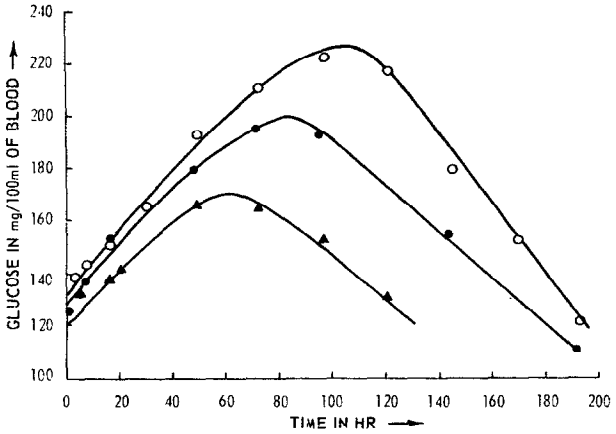

Fig. 1. Shows the changes in blood glucose concentration (mg/100 ml blood) after the administration of triamcinolone diacetate to rats of 2.5 mg/100 g body weight (△–△), 5 mg/100 g body weight (●–●) and 10 mg/100 g body weight (○–○) and sacrificed at different hr after the administration of the hormone. Control animals received saline injection instead of the hormone at the time of the latter's administration. Each result represents an average of results obtained with 10 animals.

returned to normal or near normal levels 125, 170 and 190 hr after the administration of 2.5, 5 and 10 mg per 100 g of body weight of hormone. The changes in ribonuclease activities measured at pH 5.8 (upper curves) and pH 7.6 (lower curves) after the administration of two different doses of hormone (2.5 mg and 5.0 mg per 100 g body weight) are shown in fig. 2. A decrease in the activities of acid (pH 5.8) and alkaline (pH 7.6) ribonucleases commenced immediately after the administration of the hormone. The decrease in activities was gradual and continued to a minimum of 40–45% in the case of acid ribonuclease, and 60–70% in the case of alkaline ribonuclease respectively of the original activities, and 60 and 70 hr respectively after the administration of the hormone. This was followed by a gradual increase to normal levels. It is apparent from fig. 2 that the shapes of the activity curves noted after the administration of two different doses of hormone were very much alike.

The changes in total liver RNA and the activities of aspartate and alanine aminotransferases after hormone administration (2.5 mg/100 g body weight) are shown in figs. 3 and 4. Fig. 3 indicated that the pattern of changes in liver RNA was very much similar to the one observed with glucose production (see fig. 1). The liver

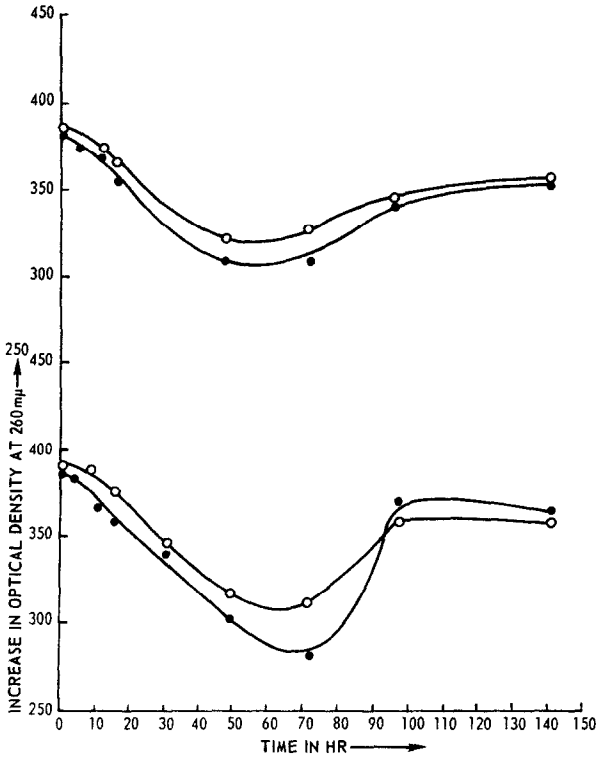

Fig. 2. Shows the changes in ribonuclease activity at pH 5.8 (upper curves) and pH 7.6 (lower curves) after the administration of triamcinolone diacetate to rats of 2.5 mg/100 g body weight (○–○) and 5 mg/100 g (●–●) body weight and sacrificed at different hours after the administration of the hormone. The reaction mixture in 1 ml contained 20 μmoles acetate buffer pH 5.0 (for acid ribonuclease activity measurement) or tris-HCl buffer pH 7.6 (for alkaline ribonuclease activity measurement), 2 mg RNA, and 1.5 mg enzyme protein of mitochondrial suspension. This mixture was incubated for 1 hr at 37°C and terminated by adding 2 ml of a cold 0.6 N perchloric acid ($HClO_4$), cooled, and then centrifuged. 1 ml of the supernatant was diluted to 3 ml with water and the optical density (OD) at 260 mμ was measured against a blank containing the same percentage of $HClO_4$. The results are arbitrarily expressed as the difference between the experimental and blank. Control animals received saline injection instead of the hormone at the time of the latter's injection. Each result represents an average of results obtained with 10 animals.

RNA which showed a regular increase from 8.2 to 12.6 mg (per g of liver tissue) in the first 48 hr after hormone administration, declined slowly until reached the normal level. The maximum value attained was 50% higher than the control. A 40% increase

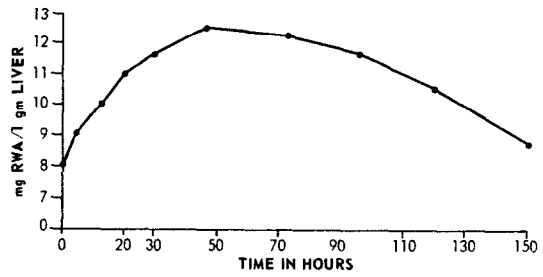

Fig. 3. Shows the changes in the liver RNA after hormone administration. The animals received 2.5 mg of triamcinolone diacetate per g body weight and were sacrificed at different hours after the administration of the hormone. Each result represents an average of results obtained with 5 different animals. Control animals received saline injection instead of the hormone at the time of the latter's administration.

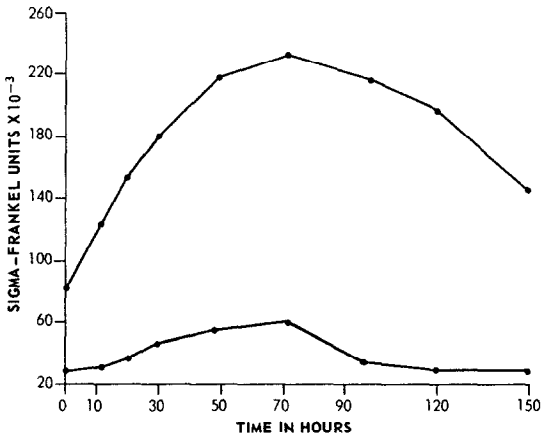

Fig. 4. Shows the changes in the activities of hepatic aspartate and alanine aminotransferases in the high-speed supernatant fractions from the liver of rats which received 2.5 mg of triamcinolone diacetate per 100 g body weight and were sacrificed at different hours after the administration of the hormone. The results are expressed in sigma units (Sigma Bulletin No. 505) per ml of the supernatant fraction. Each result in the fig. represents an average of results obtained with 5 different animals. Control animals received saline injection instead of the hormone at the time of the latter's administration.

Table 1

Shows ribonuclease inhibitor activity in the high-speed supernatant fraction from hormone-treated rat liver. The reaction mixture in 1 ml contained 20 μmoles of tris-HCl buffer pH 8.0, 2 mg RNA, 2.5 μg ribonuclease, and 1.25 mg protein of the supernatant fraction. The mixture was incubated for 30 min at 37°C and terminated by adding 2 ml of a cold 0.6 N $HClO_4$, cooled, and then centrifuged. 0.1 ml of the supernatant was diluted to 3 ml with water and the optical density (OD) at 260 mμ was measured against a blank containing the same percentage of $HClO_4$. The results are arbitrarily expressed as the difference in optical density between the experimental and blank. Animals received triamcinolone diacetate 5 mg/100 g body weight 16 and 48 hr prior to sacrifice. Each result represents an average of results obtained with 10 different animals. Control animals received saline injection instead of the hormone at the time of latter's administration.

Addition of soluble extract from the liver of	Changes in optical density	Inhibition in %
No addition	0.394	0.0
Control rat	0.191	51.5
Hormone-treated rat (administered 16 hr prior to sacrifice)	0.185	53.1
Hormone-treated rat (administered 48 hr prior to sacrifice)	0.194	50.7
Chicken	0.349	11.2

in liver RNA was also previously noted by Feigelson and Feigelson [16] in rats after cortisone administration.

The shape of the curve showing the changes in the activity of aspartate aminotransferase was also similar to one previously noted with glucose production although not identical. (Compare fig. 1 with fig. 4 upper curve.) It is to be noted that the activity of the aspartate aminotransferase was more markedly affected than that of the alanine aminotransferase after hormone administration. The changes in the activity of the latter enzyme also followed the same pattern observed in the case of glucose production or liver RNA.

The results in table 1 show the inhibition of ribonuclease activity at pH 8.0 by the high-speed supernatant fractions prepared from untreated and hormone-treated rat livers. The inhibition observed in each case was 50%.

4. Discussion

The production of blood glucose from amino acids or pyruvate is the end result of a series of reactions catalyzed by enzymes unique to gluconeogenesis [2,3]. The marked increases in glucose production observed in rats after hormone administration is believed to be caused by *de novo* synthesis of gluconeogenic enzymes [2]. Protein synthesis depends, among many factors, on the stabilities of polysomes and RNA (particularly mRNA). Since RNA stability is inversely related to ribonuclease activity, an inverse relationship between glucose production and ribonuclease activity is expected to be found in rats after hormone administration. An inverse relationship was indeed found between glucose production and ribonuclease activity after hormone administration (compare fig. 1 △—△ with fig. 2 ○—○). Furthermore, blood glucose level reached the normal when the activities of the enzymes returned to control levels. That the changes in the ribonuclease activity (fig. 2 ○—○) and glucose production (fig. 1 ○—○) were more marked after the administration of a larger dose of hormone (5 mg/100 g body weight) appears to support the conclusion. The findings of Barnabei and Ottolenghi [19] which has just been published also indicate an inverse relationship between liver nuclear RNA polymerase and ribonuclease activity after cortisone treatment. Karlson and his associates and Mandel and co-workers, on the other hand, found no change in RNA polymerase (soluble form) activity after cortisol administration.

The results presented in this paper also show an inverse relationship between ribonuclease activity and liver RNA. Relationship between RNA synthesis and ribonuclease activity on one hand and protein synthesis and ribonuclease on the other hand, were also demonstrated by Homoki et al. [17] and Siler and Fried [7]. Homoki et al. [16] observed inhibition of ribonuclease activity and stimulation of RNA synthesis by 0.4 $(NH_4)_2SO_4$ in a system involving the use of isolated rat liver nuclei as the source of the respective enzymes. Siler and Fried [7] noted much less incorporation of C^{14} amino acid into proteins (only 30% compared to that observed with similar preparations from rat liver) in a cell-free amino acid incorporating system from chicken liver. This was attributed to the uncontrolled action of ribonuclease because ribonuclease inhibitor, which has been found in rat liver [18] and is believed to regulate ribonuclease activity, was absent from chicken liver. The suppression of ribonuclease activity noticed after hormone administration could not be accounted for due to increased ribonuclease inhibitor activity since this activity was not affected after hormone administration (table 1).

The changes in the activities of hepatic aspartate and alanine amino-transferases observed after hormone administration also appeared to be related to the changes in ribonuclease activity. These enzymes participate in the initial stage in the reaction sequences of gluconeogenesis. The conclusion that glucose production is inversely related to ribonuclease activity in hormone-treated rats comes from the results presented in this paper. From the above discussion it can possibly be concluded that one of the many actions of corticosteroids is their action on ribonuclease activity, which might be considered to be indirectly responsible for marked stimulatory effect of these hormones on enzyme induction, the end result of which is reflected in an increased glucose production. Whether or not the suppression of ribonuclease activity can be attributed to decreased catabolism of enzyme protein is not certain and is now under investigation. It should be strongly emphasized, that in the undertaking of the effects of hormones the action of degradative enzymes cannot be ignored.

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