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ON TWO PARADOXICAL SIDE-EFFECTS OF PREDNISOLONE IN RATS, RIBOSOMAL RNA BIOSYNTHESES, AND A MECHANISM OF ACTION

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Abstract—Liver enlargement and muscle wastage occurred in Wistar rats following the subcutaneous administration of prednisolone. In the liver both the content of RNA and the biosynthesis of ribosomal RNA increased while both the RNA content and ribosomal RNA biosynthesis were reduced in the gastrocnemius muscle. It is suggested that the drug acted in a selective and tissue-specific manner to enhance ribosomal RNA synthesis in the liver and depress such synthesis in the muscle. This view supports the contention that the liver and muscle are independent sites of prednisolane action.

Much recent research with prednisolone (Δ′ hydrocortisone) has been concerned with enhancing the efficacy of the drug as an anti-inflammatory agent and with improving its speedy translocation to the desired site of action [1–4]. The side-effects [5] of prednisolone remain poorly understood, however, and limit the beneficial usage of the drug. Two examples of such side-effects, liver enlargement and muscle wastage, are paradoxical, were believed to be systemic and were linked to corticosteroid-induced hepatic gluconeogenesis in which the primary stimulation of hepatic protein synthesis created a demand for muscle-derived metabolites [6]. However, the detection of prednisolone-binding proteins in the cytosols of rat liver and gastrocnemius muscle [7] suggests that these two tissues may be independent sites of prednisolone action and that the mechanisms underlying the prednisolone-induced responses in the two tissues reside in the tissues. The possibility that prednisolone elicited the observed responses by interfering with RNA polymerases and ribonucleases has received some attention [8] and, here, the effect of prednisolone treatment on the rates of ribosomal RNA biosynthesis in the rat liver and gastrocnemius muscle are considered.

MATERIALS AND METHODS

Male Wistar of body weights ranging between 140 g and 190 g were used in the study. They were housed individually in the same room, fed 12 g Diet 41B containing 14% (w/w) dried skimmed milk (North Eastern Agricultural Co-operative Ltd., Bucksburn, U.K.) daily and allowed water *ad libitum*.

"Control" and "test" animals were paired on the basis of their initial fasted body weights. All animals received intraperitoneal injections of a single dose of 20 μCi of tritium-labelled orotic acid, (5-^{3}H) orotic acid, obtained from Radiochemical Centre (Amersham, U.K.). Two days later, prednisolone treatment commenced and prednisolone acetate was administered daily over the next eight days by subcutaneous injections into the hind limbs of the test group of animals, at a dose level of 1.0 mg per 100 g initial body weight. The control group received the same volume of physiological saline by the same route. Every other day, a test animal and its control partner were sacrificed and livers and gastrocnemius muscles (from a hind limb) were excised rapidly, weighed, and frozen in liquid nitrogen. Six test and six control animals were studied in replicate experiments.

Intact and undegraded cytoplasmic RNA was extracted from the gastrocnemius muscle using a phenol–chloroform mixture as previously described [9]. The extract was dissolved in 0.01 M sodium acetate buffer, pH 6.0 and passed through a column of oligo (dT)-cellulose to remove polyadenylated species of RNA. The effluent from the column was subjected to sucrose gradient centrifugation to fractionate ribosomal RNA [10] which was precipitated overnight with 2 vol. of absolute ethanol at 15°. Intact ribosomes were isolated from the liver essentially as described by Hirsch and Hiatt [11]. To obviate contamination by pool nucleotides, the ribosomal pellet was resuspended in an aqueous medium containing 0.001 M $MgCl_2$, 0.025 M KCl, 0.05 M Tris–HCl buffer, pH 7.5 at 20° using a Potter-Elvehjem homogeniser and, following centrifugation at 600 *g*, reprecipitated from the supernatant by raising the Mg^{2+} concentration of the medium to 0.1 M.

The RNA content of the sample was estimated by ultraviolet absorption spectrophotometry, at 260 nm, of the hot 0.2 perchloric acid extracts [12]. Yeast RNA served as standard for quantitation.

Suspensions of the gastrocnemius muscle ribosomal RNA and the liver ribosomes in 0.1 M KOH were loaded on glass fibre discs and counted for radioactivity on the Inter-technique liquid scintillation spectrometer (Model SL30). By plotting the logarithm of the radioactivity associated with the ribosomal RNA against a linear time scale, it was possible to obtain a straight line with a slope of K_d/

2.303, where K_d is the RNA degradation constant [13]. The biological half-life of the RNA was calculated from the degradation constant using the relationship $t_{\frac{1}{2}} = 0.693/K_d$, and the rate of ribosomal RNA biosynthesis was deduced from these data as described by Enwonwu *et al.* [14].

Protein estimations were based on the Lowry method [15].

RESULTS

Data presented in Fig. 1 confirm that, over the experimental period, prednisolone-treated rats suffered a loss of gastrocnemius muscle mass. This loss averaged about 20% when compared with the muscle mass of the control animal and became more pronounced as prednisolone treatment progressed. In contrast, the liver of these animals enlarged, with weight increases of about 15% of the control values all through the experimental period.

A significant loss of gastrocnemius muscle RNA occurred as a result of prednisolone treatment (Table 1) and, eight days after the initial prednisolone administration, the tissue lost nearly 50% of its RNA content. Liver RNA, on the other hand, increased (Table 1). This increase was statistically significant two days after the commencement of prednisolone treatment and, by day 8, was 20% more than the RNA content in the liver of untreated animals.

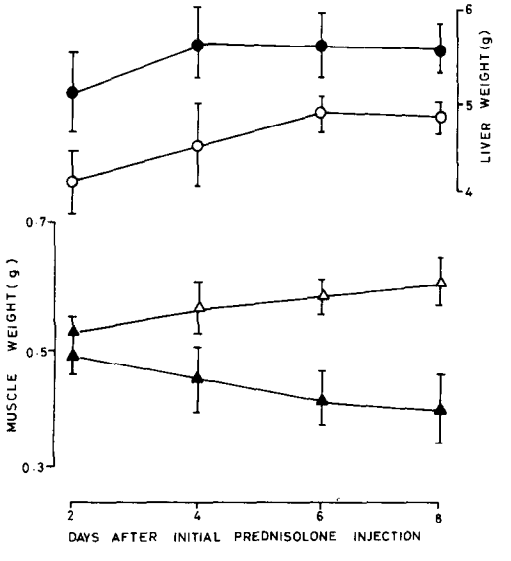

Fig. 1. The effects of prednisolone on rat liver and gastrocnemius muscle weights. Prednisolone-treated animals received subcutaneous injections of prednisolone acetate daily at a dose rate of 1.0 mg per 100 g initial body weight and the control group received the same volume of saline. Results shown are mean values from 6 rats ± S.D. Solid and open symbols indicate results from prednisolone-treated animals and corresponding controls respectively. In the muscle, the observed changes were statistically significant ($P < 0.005$) from day 4 to day 8 and in the liver, the changes were significant ($P < 0.025$) from day 2 to day 8.

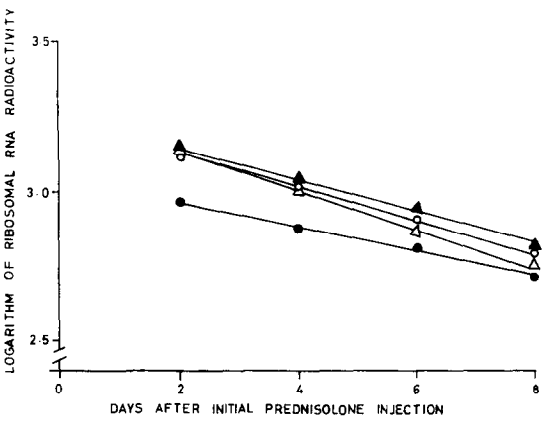

Fig. 2. Loss of activity from radiolabelled rat gastrocnemius muscle and liver ribosomal RNAs during eight days of prednisolone treatment. ^{3}H-orotic acid was administered to all animals 48 hr before commencement of prednisolone treatment. Prednisolone-treated animals (solid symbols) received prednisolone acetate subcutaneously daily at a dose rate of 1.0 mg per 100 g initial body weight and controls (open symbols) received the same volume of saline. Liver results are represented with triangles and the maximum initial labelling attained (in cpm/liver/100 g initial body weight $\times 10^3$) was 1598 ± 50. Muscle results are represented with circles and the maximum initial labelling attained (in cpm/muscle/100 g initial body weight) was 1442 ± 78. Each point on the graph is the mean result from six animals and the slope of each line was plotted by the "least squares" method. Common logarithms were used in the plots.

Figure 2 contains a plot of the logarithm of the total radiactivity associated with gastrocnemius muscle ribosomal RNA against a linear time scale. In the control animal there was a proportionate fall in radioactivity with a rate constant (K_d) of ribosomal RNA degradation of 0.14 (the product of the slope of the regression line and 2.303) and a biological half-life ($0.693/K_d$) of 4.8 days. Similar analyses of the results obtained with prednisolone-treated animals showed a ribosomal RNA degradation constant of 0.10 with biological half-life of 7.1 days. The amount of RNA synthesized per day will be equal to the amount broken down per day (calculated from the degradation constant [14]) plus the daily change in the RNA concentration. Over the experimental period, 0.16 mg of ribosomal RNA per muscle per 100 g initial body weight was synthesized per day in the control animals. The corresponding value in prednisolone-treated animals was 0.03 mg. Thus a result of prednisolone administration to rats was a marked decrease in the rate of ribosomal RNA biosynthesis in the gastrocnemius muscle. In the liver (Fig. 2), it was calculated that, with a rate constant of ribosomal RNA degradation of 0.12 and a biological half-life of 5.9 days, 8.8 mg of ribosomal RNA were synthesized per day over the experimental period in the prednisolone-treated animals. Corresponding values in untreated animals were 0.16, 4.3 days, and 6.1 mg, indicating that prednisolone caused enhanced synthesis of ribosomal RNA in the liver.

Table 1. Changes in body weight, tissue protein and RNA content of rat gastrocnemius muscle and liver following eight days of prednisolone treatment

	Day 2		Day 4		Day 6		Day 8	
	Prednisolone-treated	Control	Prednisolone-treated	Control	Prednisolone-treated	Control	Prednisolone-treated	Control
% Loss or gain in initial body weight	−0.89 ± 2.0	+5.0 ± 1.9 (<0.005)	−5.4 ± 3.0	+8.4 ± 1.9 (<0.005)	−5.5 ± 4.0	+16.7 ± 3.4 (<0.005)	−7.9 ± 3.4	+16-1 ± 4.1 (<0.005)
Muscle protein content (mg/muscle/100 g initial body weight)	82.5 ± 12.1	91.0 ± 12.6 (N.S.)	74.4 ± 8.0	99.8 ± 17.1 (<0.01)	68.9 ± 3.1	109 ± 6.4 (<0.005)	62.5 ± 6.4	108 ± 6.7 (<0.005)
Muscle RNA content (mg/muscle/100 g initial body weight)	0.93 ± 0.16	1.05 ± 0.15 (N.S.)	0.72 ± 0.06	1.11 ± 0.12 (<0.005)	0.62 ± 0.08	1.12 ± 0.06 (<0.005)	0.57 ± 0.08	1.13 ± 0.12 (<0.005)
Liver protein content (mg/liver/100 g initial bodyweight)	722 ± 82.6	576 ± 78.1 (<0.05)	800 ± 31.2	620 ± 30.0 (<0.005)	963 ± 67.1	759 ± 60.5 (<0.025)	979 ± 60.3	760 ± 47.4 (<0.01)
Liver RNA content (mg/liver/100 g initial bodyweight)	44.5 ± 5.4	37.6 ± 5.2 (<0.05)	47.8 ± 4,7	37.7 ± 4.3 (<0.025)	46.2 ± 2.6	38.3 ± 3.8 (<0.025)	47.2 ± 2.8	37.6 ± 1.6 (<0.01)

Prednisolone-treated animals received subcutaneous injections of prednisolone acetate daily at a dose rate of 1.0 mg per 100 g initial body weight and the controls received the same volume of saline. Results presented are mean values from 6 rats ± S.D. Figures in brackets indicate level of statistical significance (Student's group *t*-test) between treated and control animals. N.S. = not statistically significant.

DISCUSSION

Earlier investigations [5] established that the enlargement of the liver and the muscle wastage in prednisolone-treated rats were accompanied by parallel changes in the protein content of the two tissues. The results presented here (Table 1), which show alterations in the RNA content of these tissues, suggest that prednisolone altered the protein status of the tissues by interfering with apparatus for protein synthesis. The subsequent turnover studies showed that the biosynthesis of ribosomal RNA was enhanced in the liver and diminished in the gastrocnemius muscle, following prednisolone treatment. It was unlikely that prednisolone treatment distorted the initial RNA labelling process since it was administered two days after the tritium-labelled orotic acid, by which time maximal labelling of the RNA would have been achieved [16]. In interpreting these results, consideration was also given to possible re-utilization of labelled RNA breakdown products and prednisolone-mediated effects on the free nucleotide pool in the tissues [10]. However, no significant differences in the pattern of loss of radioactivity from the free nucleotide pool was detected between control and prednisolone-treated animals in the tissues under study. A more direct effect of prednisolone on ribosomal RNA synthesis was, therefore, indicated. Ribosomal RNA, like other cytoplasmic RNA species, originates from nuclear DNA transcription and prednisolone could act at this nuclear level to alter the rates of biosynthesis of the ribosomal species.

A major advance in the understanding of mechanisms of transcriptional control of RNA production has been the discovery of multiple forms of nuclear DNA-dependent RNA polymerase [17, 18]. These enzymes countrol genetic expression by specific recognition and transcription of different regions of DNA. Mg^{2+}-activated RNA polymerase, the enzyme responsible for the biosynthesis of ribosomal RNA, is located in the nucleolus, and we have shown [8] that prednisolone raised the activity of this enzyme in the liver but depressed it in the muscle. Thus, prednisolone may act at this nuclear level to induce or repress, in a selective and tissue specific manner, the biosynthesis of ribosomal RNA. Given the high complexity and architecture of eukaryotic ribosomes [19, 20], control of phenotypic response by regulation of ribosomal RNA synthesis would be a reasonable proposition. A chain of events regulated by prednisolone at the level of the transcription, manifesting itself in the altered rates of ribosomal RNA synthesis and, therefore, in altered rates of protein production, would account for the observed liver enlargement and muscle wastage.

The findings from the study of Mg^{2+}-activated RNA polymerase [8] and those which showed that physico-chemically different prednisolone-binding proteins existed in the rat liver and muscle [7] would, considered with the results from the present study, explain the paradoxical nature of the responses to prednisolone in the two tissues. Thus, although prednisolone acted by altering ribosomal RNA biosynthesis in both tissues, it was bound to different proteins and, therefore, translocated to different regions of the DNA template. On this template and presumably by modulating RNA polymerase activities, it promoted ribosomal RNA synthesis in the case of the liver but curbed any such synthesis in the gastrocnemius muscle. This view would support the contention that the sites of prednisolone action in the liver and muscle exist independently and that the responses of the two tissues to the drug are also independent.

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