Glucocorticoid and Mineralocorticoid Effects in Rat Liver and Kidney

Glucocorticoid effects on rat liver tyrosine amino transferase and mitochondrial enzyme activities have been associated separately with the saturation of two types of corticosterone-binding sites[1,2]. Similarly mineralocorticoid effects in toad bladder[3] have been associated with two types of aldosterone-binding sites. It has been suggested that the role of two receptors in the mechanism of steroid hormone action may be to allow control of protein synthesis at the transcriptional and translational level[4]. The present work has involved a study of the effect of actinomycin D on corticosterone-stimulated tyrosine amino transferase and mitochondrial enzyme activities in rat liver and kidney. Mineralocorticoid effects have been studied by measurement of these enzyme activities in kidneys taken from animals on high and low Na^+ diets.

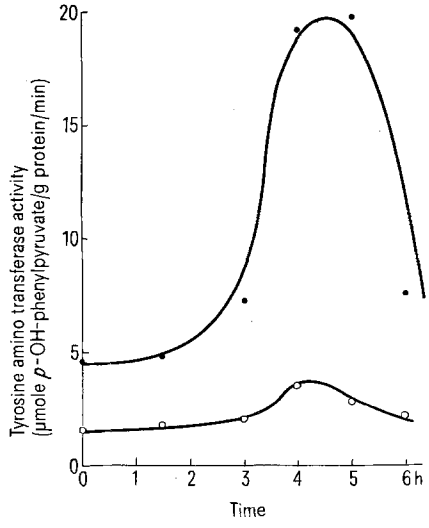

Fig. 1. Tyrosine amino transferase activity (μmole *p*-OH-phenylpyruvate/g protein per min) measured in rat liver (–●—●–) and kidney (–○—○–) homogenates taken from adrenalectomized animals at various times after i.p. injection with 5 mg corticosterone. Each point represents the mean of 2 experiments on pooled tissue (4 animals).

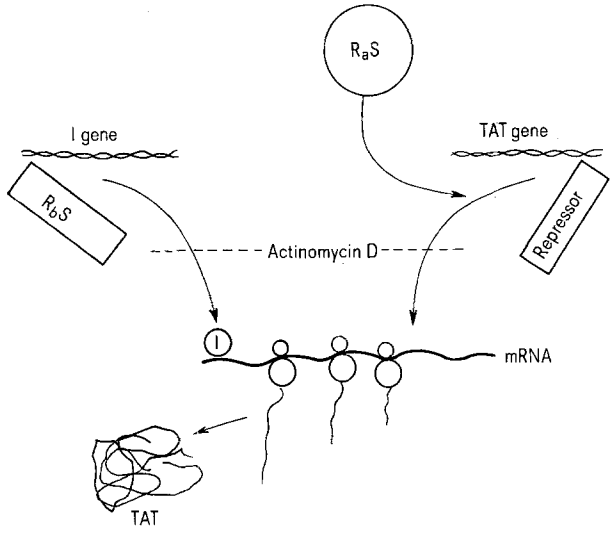

Fig. 2. The role of 2 receptors in the hormonal control of protein synthesis at the transcriptional and translational levels.

Materials and methods. 6 adult male Wistar rats were adrenalectomized and after 4 days 2 pairs were i.p. injected with 5 mg corticosterone. 1 pair of these animals was also injected, after 3 h, with 0.5 mg actinomycin D and all 6 animals killed 5 h after the hormone injection. Succinate dehydrogenase and tyrosine amino transferase activities were measured, as described previously[2], in homogenates (pH 7.4) of pooled liver or kidney tissue taken from each pair of animals. Citrate synthase was measured[5] after isolation of mitochondria by centrifugation of tissue homogenates at 10,000 rpm in an MSE 18 centrifuge. The mitochondria were twice washed and resuspended in 50 m*M* phosphate buffer (pH 7.4) then subjected, at 0 °C, to a 30 sec burst in an MSE 100 watt ultrasonic disintegrator, at maximum strength. Samples were assayed for citrate synthase activity and protein concentration using standard techniques. This experiment has been repeated 6 times.

In order to distinguish mineralocorticoid and glucocorticoid effects in the kidney the enzyme activities were measured in kidneys taken from a group of untreated animals or from animals kept on high or low Na^+ diets

Succinate dehydrogenase, SDH (E_{490}/mg protein per h), tyrosine amino transferase, TAT (μmole *p*-OH-phenylpyruvate/g protein per min) and citrate synthase, CE (μmole citrate/g protein per min) activities assayed at t = 5 h in tissue taken from adrenalectomised rats (control group 1) injected i.p. with corticosterone (5 mg) at time zero (groups 2 and 3) and with actinomycin D (0.5 mg) at t = 3 h (group 3)

		SDH	TAT	CE
Liver	1.	10.7 (12)	4.5 (12)	104 (12)
	2.	16.1 (12)	24.5 (12)	105 (12)
	3.	13.0 (12)	26.8 (12)	100 (12)
Kidney	1.	14.4 (8)	1.4 (8)	166 (10)
	2.	18.2 (8)	3.6 (8)	234 (10)
	3.	14.5 (8)	1.9 (8)	191 (10)

Enzymes assayed in tissue obtained from normal rats (group 1), from rats on a low Na^+ diet (group 2) and from rats on a high Na^+ diet (group 3) injected i.p. with aldosterone 5 h before assay (group 4)

		SDH	TAT	CE
Kidney	1.	7.9 (8)	1.4 (8)	161 (8)
	2.	9.0 (8)	2.4 (8)	235 (8)
	3.	9.8 (8)	0.7 (8)	170 (8)
	4.	7.8 (8)	1.1 (8)	176 (8)

The results represent the mean value of our results obtained as described in text on 8–12 animals for each experiment.

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for 10 weeks. This experiment has been carried out using pooled tissue from paired animals and has been repeated 5 times.

The time course for corticosterone-stimulation of tyrosine amino transferase activity in rat liver and kidney has been measured in tissue obtained from pairs of adrenalectomised rats, killed at various times during a 6-h period following a 5 mg corticosterone injection. This experiment has been repeated.

Results and discussion. The time course for corticosterone-stimulated tyrosine amino transferase activity (Figure 1) confirms a maximum activation of the enzyme during the 4–5-h period following hormone treatment[6]. There was no hormone effect on citrate synthase activity in rat liver (Table) but a characteristic glucocorticoid-stimulation of succinate dehydrogenase and tyrosine amino transferase activity.

The apparent succinate dehydrogenase activation[2] by corticosterone is believed to reflect an increased redox state in the mitochondria, which in liver may be associated with increased fat oxidation. The failure of actinomycin D to superinduce this enzyme activity is in contrast to the superinducible tyrosine amino transferase activity. This result is taken to support the suggestion[4] that the highest affinity binding sites for corticosterone in rat liver may be responsible for all transcriptional events associated with the hormone action (Figure 2), whereas the basic corticosterone-receptors may be associated with the translational control of key gluconeogenic enzymes, acting as repressors of post transcriptional inhibitors[6]. Increased fat oxidation in the liver may have a permissive role in the gluconeogenic effect of corticosterone, giving rise to key intermediates that exert secondary control of gluconeogenic pathways.

In rat kidney there is evidence of corticosterone-activation of all 3 enzymes studied which are sensitive to actinomycin D inhibition. These enzyme activities compare with those measured in kidneys taken from animals on a low Na^+ diet. In particular the citrate synthase activity corresponds with aldosterone-stimulated levels[7]. As there was no stimulation of this enzyme in liver it is probable that this effect of high concentrations of corticosterone in kidney is mineralocorticoid.

The observed loss of a mineralocorticoid response in kidneys taken from animals on a high Na^+ diet may be understood in terms of the 'escape phenomenon'. The cause of this effect is not understood but it is of interest to note that in recent work[8] a normal hormone response has been re-established following treatment with prolactin or oxytocin.

Zusammenfassung. Die biochemischen Wirkungen von Aldosteron in der Rattenniere und Corticosteron in der Rattenleber wurden untersucht und der Mechanismus der Hormonwirkung diskutiert.

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Protein Biosynthesis and Hyperpolarization of Cells

The main idea of this report is to show that one of the most important mechanisms regulating both the membrane potential level of cell and the development of hyperpolarization is connected with protein biosynthesis. In this respect, 2 groups of facts dealt with in this report will be relevant: a) activation of protein biosynthesis leads to the development of cell membrane hyperpolarization; b) inhibitors of protein biosynthesis prevent the development of hyperpolarization.

Methods. The experiments were carried out on 120 white rats aged 8–10 months. The narcosis, urethane 0.1 g/100 g body weight, was used. The membrane potential of liver cells and muscle fibres (m. gastrocnemius, m. gracilis) was determined by method of intracellular measurement using a glass 1 μm tip diameter microelectrode[1]. The RNA content was determined by the orcinol or spectrophotometric method[2]. In order to study the RNA synthesis, the solution of C^{14}-adenine 30 μCi per 100 g body weight, or $Na_2HP^{32}O_4$ 30 μCi/100 g, were i.p. injected to animals 1 h before killing. Radioactivity was expressed in imp/min/mg RNA. Protein was estimated according to method of LOWRY et al.[3]. For electron microscopic studies, liver samples were treated by glutaraldehyde and OsO4 in cold and then embedded into epon 812. The JEM-100 electron microscope with magnification 40,000 was employed.

Results and discussion. Activation of protein synthesis in liver cells and single muscle fibres was achieved in different ways. It was shown sharp activation of protein synthesis in liver cells occurred following the bloodletting. Furthermore, it enabled the restoration of serum protein content. As indicated in Figure 1, already 12 h after bloodletting (2–3% of the body wt.) one could observe a rise in membrane potential level of liver cells as well as in RNA and protein contents. A rise was also observed in the RNA synthesis intensity. Thus, if before bloodletting, the RNA renovation rate was 928 ± 84 imp/min/mg RNA, 24 h after bloodletting it equalled 1730 ± 104 imp/min/mg RNA.

The development of cell membrane hyperpolarization and activation of protein biosynthesis coincide with obvious structural changes revealed electron-microscopically. Increased in liver cells were found the numbers of membranes of endoplasmatic net, of ribosomes as well as those of nuclei per cell (Figure 2).

Analogous findings were obtained at activation of protein biosynthesis in liver cells occurring during their regeneration. It was found that the extirpation of 2/5 of liver leads to the increase in membrane potential and RNA renovation (Figure 3).

Then, a number of hormones, as genetic inductors, can markedly effect the processes of protein biosynthesis[4,5]. As is seen in Figure 4A, rats injected by insulin (0.16

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