EFFECT OF HYPOPHYSECTOMY AND CORTICOTROPIN REPLACEMENT ON STEROL ESTER HYDROLASE ACTIVITY FROM RAT ADRENAL GLAND

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1. Introduction

It has been well documented that, apart from the stimulation of steroid hormone synthesis in the adrenal gland within a short period of time, corticotropin (ACTH) maintains the activity levels of enzymes involved in steroid hormone synthesis over a longer period of time [1].

Evidence has been presented that hypophysectomy results in a rapid decrease in the activity of the cholesterol side-chain cleavage enzymes [2–4], 21-hydroxylase, 11β-hydroxylase, and also the concentration of cytochrome *P*-450 and iron–sulphur protein (adrenodoxin) in rat adrenal cortex [4].

By contrast the total protein and the activities of enzymes not directly involved in steroid hormone synthesis such as succinate, isocitrate and glucose-6-phosphate dehydrogenases do not decline so rapidly after hypophysectomy [3]. Corticotropin injection to hypophysectomized rats causes an increase in the activities of the hydroxylases involved in steroid hormone synthesis [2,3], and also in the concentration of cytochromes *P*-450 and iron–sulphur protein in rat adrenals [4]. The main source of substrate for steroid hormone synthesis in the rat adrenal gland is cholesterol esters stored in the lipid droplets [5]. It has been postulated that increased hydrolysis of cholesterol esters in the adrenal gland after ACTH injection to hypophysectomized rats [6], has been due to increased activity of sterol ester hydrolase (EC 3.1.1.13), and that this is an important factor in the mechanism of corticotropin action [6,7]. Later it was shown that the activity of sterol ester hydrolase in the adrenal cortex can be enhanced either by ACTH administration to intact [8] or hypophysectomized [9] rats, or by increasing the concentration of corticotropin in blood by the means of ether stress [7]. A decrease in adrenal sterol ester hydrolase activity 24 h after hypophysectomy has also been reported [9], but the dynamics of sterol ester hydrolase decay following hypophysectomy, and of the increase in activity of the enzyme by corticotropin replacement to such animals, have not been reported.

The aim of this work was to estimate the dynamics of the decay of sterol ester hydrolase activity in the adrenal glands of male hypophysectomized rats, and to evaluate the effect of ACTH replacement on the activity of this enzyme.

2. Materials and methods

2.1. *Chemicals and radiochemicals*

Porcine corticotropin (Corticotropin Zinc) was obtained from Organon Laboratories, Morden. Sephadex G-25 was a product of Pharmacia, Uppsala. Cholesterol oleate (99% pure), oleic acid (99% pure), corticosterone and bovine serum albumin fraction V were purchased from Sigma Chemical Co., St Louis. The [4-^{14}C]cholesterol (spec. act. 50 mCi/mmol) and [1α,2α(*n*)^{3}H]cholesterol (spec. act. 40–60 Ci/mmol) were obtained from The Radiochemical Centre,

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Amersham, and were purified by thin-layer chromatography on silica gel G (Merck, Darmstadt) before use The [4-^{14}C]cholesterol oleate was synthesized and purified as in [11] Other reagents were obtained from Koch-Light, Colnbrook and were of analytical grade

2 2 *Animals and animal treatment*

Hypophysectomized male rats of Wistar strain (~180 g) and sham-operated animals were provided by Carworth Europe, Alconbury, Huntingdon A group of hypophysectomized animals received a daily subcutaneous injection of ACTH (10 μg/100 g body wt) in 0 2 ml saline, beginning on the third day after hypophysectomy, and were killed 3 or 6 days thereafter The remainder of the hypophysectomized animals were divided into subgroups of 3 rats each and killed 3, 6 or 9 days after the operation Control, sham-operated animals, divided into appropriate subgroups, were injected subcutaneously with 0 2 ml saline daily, beginning on the third day after surgery, and killed on that day or 3 or 6 days thereafter

2 3 *Preparation of the enzyme extract*

The animals were killed by decapitation and the trunk blood was taken for the estimation of plasma corticosterone concentration [12] The adrenals were quickly removed, trimmed free of fat and weighed The glands taken from the animals of each subgroup were pooled and homogenized, by a Teflon–pestle–glass Potter-Elvehjem homogenizer, in ice-cold 0 25 M sucrose containing 0 01 M Tris–HCl buffer (pH 7 4) Aliquots were taken for estimation of the cytochrome *P*-450 concentrations in the homogenate [13] The cell debris, nuclei, mitochondria, lysosomes, microsomes and the lipid droplets were removed by centrifugation, and a 105 000 × *g* supernatant, containing 70% of the total sterol ester hydrolase activity [10], was chromatographed on Sephadex G-25 column to remove low molecular compounds and ions [14] The procedure was carried out at 4°C

2 4 *Assay of sterol ester hydrolase activity*

The reaction mixture contained in 0 5 ml final vol 0 05 M Tris–HCl buffer (pH 7 4), 25 mM KCl, 2 5 mM 2-mercaptoethanol, 60 μM [4-^{14}C]cholesterol oleate in 5 μl acetone, [^{3}H]cholesterol to correct for losses during the procedure, and 105 000 × *g* supernatant of the adrenal homogenate, containing 0 25–0 5 mg protein In these conditions the reaction rate was linear over the incubation period and range of the protein concentration employed The reaction was carried out for 30 min at 37°C and was terminated by adding 4 5 ml ethanol acetone mixture (1 1, v/v) The liberated, radioactive cholesterol was separated and counted as in [10] Protein concentration was determined by the Lowry method [15] with bovine serum albumin being the standard The results were statistically evaluated by Student's *t*-test [16]

3 Results

3 1 *Effect of hypophysectomy on cytochrome P-450 concentration in the homogenates of the adrenal glands*

The concentration of cytochrome *P*-450 in the adrenal homogenates exponentially decreased after hypophysectomy When the logarithm of cytochrome *P*-450 concentration was plotted against time after

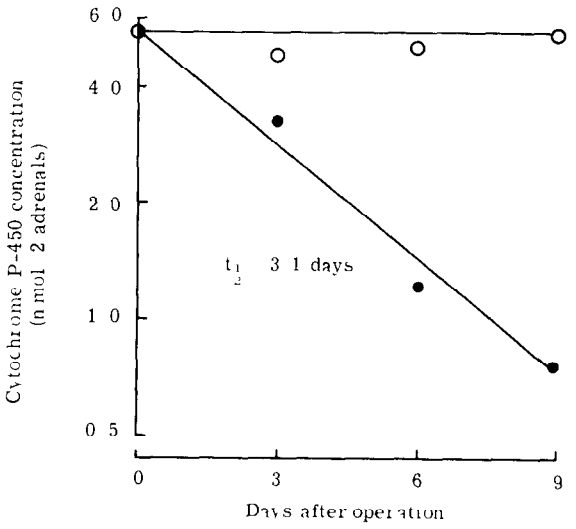

Fig 1 Time course of cytochrome *P*-450 decay in the homogenate of male rat adrenal glands after hypophysectomy or sham operation The concentration of cytochrome *P*-450 was measured as in [13] from the CO + dithionite versus CO difference spectra, using ε = 100 cm^{-1} mM^{-1} at 450–490 nm The measurements were plotted against time on a semi-log scale Half-life ($t_{1/2}$) of cytochrome *P*-450 was calculated (○–○) sham operation, (●–●) hypophysectomy

hypophysectomy a straight line was obtained and from this the biological half-life of the cytochrome *P*-450 pool in the adrenal gland was found to be 3.1 days (fig.1). In control animals the cytochrome *P*-450 concentration did not change with time after sham operation and mean concentration amounted to 5.8 nmol/2 adrenals.

3.2. *Effect of hypophysectomy and ACTH replacement on animal body weight, the weight of the adrenal glands and serum corticosterone concentration*

Three days after the operation the body weights were the same in all groups of animals. Six and nine days after surgery body weights were significantly lower in the group of hypophysectomized or hypophysectomized and ACTH injected animals, as compared with the sham-operated controls. In the group of hypophysectomized animals 3, 6 and 9 days after the operation the weights of the adrenal glands were significantly lower than in the control group. Corticotropin injection to hypophysectomized rats resulted in a significant increase in the weight of the adrenal glands only 6 days after hormone treatment as compared with the sham-operated controls. Whereas in comparison with a group of hypophysectomized animals a significant increase in the adrenal weight was noted after daily ACTH injection for 3 as well as for 6 days. Serum corticosterone concentration was lower in rats 6 and 9 days after hypophysectomy, as compared with the sham operated animals. Corticotropin replacement resulted in a highly significant increase in serum corticosterone concentration as compared with the control group (table 1).

3.3. *Effect of hypophysectomy and ACTH replacement on adrenal sterol ester hydrolase activity*

In hypophysectomized animals the specific activity of sterol ester hydrolase decreased from the initial activity of 107.5 ± 4.8 three days after hypophysectomy to 74.0 ± 4.5 and 43 ± 2.5 pmol. min^{-1}. mg^{-1} six or nine days after the operation, respectively. Three days after the operation there were virtually no changes in the activity of the enzyme as compared with sham-operated, control animals. In sham-operated rats no changes in the specific activities of adrenal sterol ester hydrolase were detected 3, 6 and 9 days after surgery and the average specific activity of the enzyme was 105.0 ± 3.7 pmol. min^{-1}. mg^{-1} and did not differ from the level of sterol ester hydrolase activity found in the same batch of rats, not being subjected to either hypophysectomy or sham operation.

Daily injection of ACTH to the rats beginning at the third day after hypophysectomy resulted in an increase in specific activity of the enzyme to 156.0 ± 11.2 and 273.0 ± 10.8 pmol. min^{-1}. mg^{-1} three or six days after hormone injection and was significantly different from the activity observed in the appropriate groups of hypophysectomized animals, and also from the sham operated animals but only after 6 days of hormone treatment.

Table 1
Effect of hypophysectomy and ACTH replacement in male rat on body weight, adrenal weight and serum corticosterone concentration

Days after operation	Body weight (g)			Adrenal weight (mg/2 glands)			Serum corticosterone concentration (μg/dl)		
	Hypox	Sham	Hypox +ACTH	Hypox	Sham	Hypox +ACTH	Hypox	Sham	Hypox +ACTH
3	183 ± 4	195 ± 2	–	26.8 ± 0.3[a]	37.5 ± 2.6	–	3.9 ± 0.5	6.0 ± 0.1	–
6	169 ± 4[a]	218 ± 4	157 ± 4[a]	17.7 ± 0.7[c]	37.5 ± 2.8	35.6 ± 0.2	1.6 ± 0.1[a]	5.5 ± 1.2	25.2 ± 7.2[b]
9	168 ± 3[b]	226 ± 5	162 ± 4[b]	13.0 ± 0.4[c]	39.1 ± 2.3	45.5 ± 0.5[a]	1.6 ± 1.0[a]	5.9 ± 1.9	36.8 ± 1.5[a]

[a] Test versus sham $p < 0.05$; [b] test versus sham $p < 0.01$; [c] test versus sham $p < 0.001$

Corticotropin was injected daily in a dose of 10 μg/100 g body wt through 3 (row 2) or 6 days (row 3) beginning at the third day after hypophysectomy. Corticosterone concentration was estimated by the method in [12]. The results are the mean of 3 estimations ± SEM. Hypox, hypophysectomy; Sham, sham-operation

3 4 *Estimation of half-life of sterol ester hydrolase from the adrenal glands*

When the logarithm of the total activity of the enzyme was plotted against time after hypophysectomy a straight line was obtained, and from this a biological half-life of sterol ester hydrolase of 4 9 days could be calculated No changes in the total activity of the enzyme were observed in the adrenal glands of sham-operated animals (fig.2)

4 Discussion

The results obtained indicate that apart from the well known effects of lowering adrenal weight and serum corticosterone concentration (reviewed in [17]) hypophysectomy resulted in a decrease in the cytochrome *P*-450 concentration in adrenal homogenates with $t_{1/2}$ 3 1 days In the homogenates of male rat adrenals two rates of decay of cytochrome *P*-450 with a $t_{1/2}$ 2 5 days and $t_{1/2} > 20$ days were reported It was suggested that these values represent the decay rates of zona fasciculata-reticulens and zona glomerulosa cytochrome *P*-450, respectively [4] Whereas in female rat adrenals only one rate of decay with $t_{1/2}$ 3.6–3 8 days was found [4] The last data are in close agreement with $t_{1/2}$ 3 5 days reported [18] A small difference between the rate of decay of adrenal cytochrome *P*-450 obtained and reported in [4,18] might be explained by a different source or strain of rats or by different weight of the animals and also by the different proportion of zona glomerulosa cytochrome *P*-450 to zona fasciculata cytochrome *P*-450 in the preparation However in contrast to the data in [4] no appreciable changes in cytochrome *P*-450 concentration were found in sham-operated animals 3, 6 and 9 days after surgery Therefore it seems that in the present study the sham operation did not impair ACTH secretion in these animals Corticotropin replacement resulted in an increase in the adrenal weight and serum corticosterone concentration, and after 3 days of treatment the adrenal weights were the same as in the control sham-operated group, and on the sixth day exceeded the level observed in the control animals

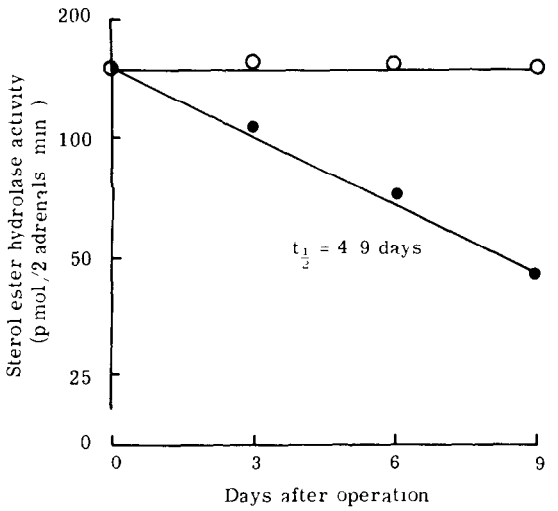

Fig 2 Time course of sterol ester hydrolase decay in a 105 000 × *g* supernatant of male rad adrenals after hypophysectomy or sham operation Preparation of the enzyme extract and measurement of the activity of sterol ester hydrolase were described in section 2 The measurements were plotted against time on a semi-log scale Half-life ($t_{1/2}$) of sterol ester hydrolase was calculated (○–○) sham operation, (●–●) hypophysectomy

Sterol ester hydrolase activity in the cytosol of the adrenal gland decreased after hypophysectomy giving linear decays on a log plot with $t_{1/2}$ 4 9 days, similar to the $t_{1/2}$ values calculated for steroid hydroxylases [2,4,18] The decay rate was more rapid than the decay of total protein for which $t_{1/2}$ 6 8–7 2 days had been reported [4] Daily injection of ACTH to hypophysectomized rats, beginning on the third day after hypophysectomy, and continued through 3 or 6 days caused an increase in the specific activity of the enzyme, the final activity being ~2 5-times higher than the initial activity observed either in control or 3 day hypophysectomized rats In sham-operated animals no appreciable decrease in the total activity of sterol ester hydrolase was observed 3, 6 or 9 days after surgery This is consistent with the estimations of weights of the adrenal glands, serum corticosterone and cytochrome *P*-450 concentration, and also with the results in [2], who found no decrease in the activity of cholesterol side-chain cleavage enzyme in the adrenal glands of sham-operated rats The results obtained in the present study suggest that the activity of sterol ester hydrolase from the adrenal gland is influenced by ACTH in a mode similar to the activity of steroid hydroxylases and their components, such as cytochrome *P*-450 and iron–sulphur protein

In the light of numerous reports [7,10,11,19–22,25] it is evident that free cholesterol side-chain cleavage enzyme is an important step in steroid hormone synthesis in the adrenal gland, and hydrolysis of cholesterol esters, catalysed by an ACTH-sensitive sterol ester hydrolase [8,11,14,21,23,24] is providing substrate for steroid hormone synthesis [5–7,14,19,22]. Therefore the increase in the activity of sterol ester hydrolase as a result of corticotropin action might be an important factor ensuring adequate supply of substrate for increased steroid hormone synthesis in the adrenal gland.

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