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CORTICOSTERONE IN THE AQUEOUS HUMOUR OF THE RABBIT EYE

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SUMMARY

Corticosterone was identified in the aqueous humour of the rabbit eye by chromatography, acetylation with [^{14}C]acetic anhydride and crystallization to constant specific activity. The level of corticosterone in aqueous humour, determined by a competitive protein binding method, was found to be approximately 10 times lower than the concentration of corticosterone in the peripheral plasma of the female rabbit.

INTRODUCTION

ATTEMPTS to determine the corticosteroids in the aqueous humour of the eye were made as early as 1952 [1, 2]; however, the findings obtained by photometric methods have not been confirmed by more specific and sensitive methods until now.

The present paper reports the identification of corticosterone and its determination by a competitive protein binding method in the aqueous humour of the rabbit eye. Rabbits are commonly used as experimental animals for the investigation of the physiology and biochemistry of the eye; therefore, the determination of the corticosterone levels in various rabbit eye structures seems to be important from the viewpoint of both steroid biochemistry and ophthalmology.

EXPERIMENTAL

Animals and sampling. Six female rabbits of the Chinchilla strain weighing 2·5–3 kg were used. The left eye of an animal was proptosed and the aqueous humour (0·15–0·25 ml) was withdrawn with a tuberculin syringe and stored. Blood samples were obtained from a short cut across the marginal ear vein. The blood was heparinized and centrifuged. After 3 h a second blood sample was obtained from the other ear. Thereafter the rabbits were sacrificed, their eyes were enucleated and aqueous humour was withdrawn from both eyes. If the anterior chamber is opened and the aqueous humour is allowed to escape, the newly formed fluid which fills the eye differs considerably in its physical and chemical properties from the normal, primary aqueous humour. This fluid has been called plasmoid or secondary aqueous humour and its greatest change is the increase in protein content. Consequently, at the second withdrawal the secondary aqueous humour was collected from the left eyes and the primary from the right ones. Plasma and aqueous humour were immediately frozen and stored at −15°C until analyzed.

Reagents. All solvents were of analytical grade and were redistilled before use. [^{3}H]Cortisol (Radiochemical Centre, Amersham, England; S.A. 30·0 Ci/mmol) was purified by chromatography on a thin layer of silica gel and on paper. Corticosterone and corticosterone acetate were purchased from Light and Co.,

Colnbrook, England. [^{14}C]Acetic anhydride (Radiochemical Centre, Amersham) had a specific activity of 45 mCi/mmol.

Chromatography. T.l.c. was carried out on silica gel (Kieselgel HF_{254}, Merck, Darmstadt) in the system dichloromethane–methanol, 97:3 (v/v). Chromatography on Whatman No. 1 paper was performed in the Bush B3 system (benzene–light petroleum/b.p. 40–60°C–methanol–water, 5:10:12:3, by vol.).

Protein binding assay. The method of Pegg and Keane[3] was used, [^{3}H]cortisol being the steroid displaced from its binding to human transcortin. In rabbit plasma, the concentration of corticosteroids was determined by the protein binding method[3] and by the fluorimetric method of van der Vies[4].

RESULTS

Identification of corticosterone in the aqueous humour of the rabbit eye

Pooled primary aqueous humour of the rabbit eye (1.5 ml) was extracted with 6 ml of dichloromethane and the organic phase was dried with anhydrous sodium sulphate and then evaporated *in vacuo*. The dry residue was submitted to t.l.c. and the corticosterone zone (R_F 0·23) was eluted and then acetylated with 2 mCi [^{14}C] acetic anhydride in 0·2 ml of pyridine–benzene (1:1, v/v) at 85°C for 30 min and then for 18 h at room temperature. The dry residue of the acetylated material was rechromatographed on silica gel in the same system and the corticosterone acetate zone (R_F 0·44) was then chromatographed on paper in the Bush B3 system. The eluted corticosterone acetate (R_F 0·68; 3024 dpm) was admixed with 8·55 mg of corticosterone acetate and crystallized to constant specific activity. The criterion of radiochemical homogeneity was met as shown in Table 1. The average concentration of corticosterone in the pooled primary aqueous humour of the rabbit eye was 6·8 ng/ml.

Table 1. Crystallization of corticosterone [^{14}C] acetate from aqueous humour of the rabbit eye to constant specific activity. Eluate after chromatography was crystallized with 8·55 mg of authentic corticosterone acetate

Crystallization No.	Crystallized from (solvent mixture)	Specific activity d.p.m./mg
1	dichloromethane–cyclohexane	329
2	acetone–cyclohexane	176
3	acetone–cyclohexane	171
4	dichloromethane–cyclohexane	173

Estimation of corticosteroids in the aqueous humour

The competitive protein binding method used[3] does not differentiate between corticosterone and cortisol. However, as the characterization of the steroid in the aqueous humour indicates, the major corticosteroid is corticosterone, as in rabbit plasma.

The corticosterone concentrations in the primary aqueous humour, aqueous humour of the same eye obtained 3 h later and from the intact contralateral eye after 3 h are given in Table 2.

Corticosterone concentration in the rabbit plasma

Samples of plasma were withdrawn at the first sampling of the aqueous humour

Table 2. Corticosterone concentration in primary and secondary aqueous humour of the rabbit eye assayed by protein binding method

Aqueous humour	No. of samples	Corticosterone ng/ml ± S.D.
primary–left eye	6	7·76 ± 4·33
secondary–left eye, after 3 h	6	4·61 ± 2·22
primary–right eye, after 3 h	6	4·37 ± 2·73

and 3 h later at the second paracentesis of both eyes. Corticosteroid determinations were carried out both by the competitive protein binding method and by fluorimetry. The results are shown in Table 3.

Table 3. Corticosteroid concentration in rabbit plasma at the 1st and 2nd (after 3 h) withdrawal of aqueous humour assayed by competitive protein binding (CPB) and fluorimetric methods

Withdrawal No.	No. of samples	CPB Corticosteroids ng/ml ± S.D.	Fluorimetric method Corticosterone ng/ml ± S.D.	Fluorimetric method Cortisol ng/ml ± S.D.
1	6	56 ± 16·5	36 ± 19·6	8 ± 4·5
2	6	59 ± 9·8	51 ± 22·1	10 ± 5·2

DISCUSSION

Corticosterone and cortisol are found in the plasma and in the body tissues of the rabbit. Their ratio is not constant: under normal conditions the concentration of corticosterone is 4–5 times higher than that of cortisol; however, under the influence of various factors the cortisol fraction increases as e.g. after ACTH [5, 6], in the early ontogenesis [7] or after changes of steroid precursors *in vitro* [8]. Corticosterone is the main corticosteroid secreted by the rabbit adrenal and circulating in the blood; however, a considerable concentration of cortisol has been found in some tissues, such as the kidneys, spleen, heart and brain [9].

In the aqueous humour of the rabbit eye, corticosterone was characterized as the main corticosteroid, its concentration being approximately 7–14 times lower than in the plasma. In earlier studies [1, 2, 10], the corticosteroids of aqueous humour of the rabbit eye were determined as Porter–Silber chromogens on the assumption that cortisol was the main corticosteroid component. These assays resulted in considerable overestimation of the corticosteroid concentration due to unspecific chromogens, similarly to the analyses using blue tetrazolium [11–15]; concentrations of, e.g. 3·4 μg/m [11] or 0·1 μg/mg of aqueous humour [12] were reported.

The concentrations of corticosterone and cortisol determined by us in rabbit plasma agree with those reported by other authors [9]. Concentrations of corticosterone in the aqueous humour of the rabbit eye may reflect the presence of a barrier retarding an unrestrained penetration of the steroids from blood to aqueous humour. The differences of the concentrations between plasma and aqueous humour was found not only in normal but also in secondary, plasmoid

aqueous humour. The ratio of concentrations of corticosterone in blood and aqueous humour in the rabbit resembles the ratio of corticosteroid concentration between blood and cerebrospinal fluid found in man[16].

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