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STEROIDS AND INTRAOCULAR PRESSURE

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SUMMARY

The intraocular pressure is dependent upon the aqueous humour formation by the ciliary body and on the outflow through the trabecular meshwork localized in the anterior chamber angle of the eye. In the ciliary body epithelium the active sodium transport has been demonstrated and effects of mineralocorticoids has been suggested but not yet equivocally confirmed. Outflow facility of the trabecular meshwork is connected with the presence of acid mucopolysaccharides. Clinical overdosage of glucocorticoids results in an increase of intraocular pressure, so-called steroid glaucoma. It is explained by the action of glucocorticoids on the stabilization of lysosomal enzymes needed for physiological decomposition of these macromolecular substances. Disbalance of oestrogen supply may lead to disorder in intraocular pressure, too.

The occurrence of glucocorticoids, mineralocorticoids, oestrogens and testosterone of endogenous origin in aqueous humour, the metabolism of these hormones and evidence for formation of steroid-receptor complexes is presented.

Persistent elevation of intraocular pressure, visual field defects and pathologic cupping of the optic discs characterize glaucoma. In the U.S.A. it is estimated that there are 2 million people with glaucoma and according to Leydhecker [1] in world scale it is the cause of each fifth blindness (in U.S.A. 14%, in USSR 22.7% and in Iceland 60–70% of all blind people).

Intraocular pressure (IOP), viewed teleogically, exists in order to maintain the eyeball in such a shape that it can function as an optical instrument. In a normal eye it is maintained in physiological limits by complex mechanisms, which, however, are not fully understood. The relation between blood and intraocular pressures are still a matter of many discussions, but according to overwhelming opinion there is not a clear-cut correlation between both physiological variables.

IOP is maintained by the equilibrium between the forces pumping the intraocular fluid, the aqueous humour, into the eye and those which tend to hinder its escape. In human and animal studies besides physico-chemical factors such as plasma pH, blood pressure, serum osmolarity etc., several hormones have been shown to affect IOP. These include prostaglandins, thyroxine, catecholamines, prolactin, ADH, MSH and steroid hormones aldosterone, glucocorticoids, oestrogens and even androgens. In a recent review Waitzman [2] concluded that the hypothalamus might be the major central nervous system controlling site for changes in IOP.

Mechanism for hormonally induced rise of IOP has not been clear until now because of the complexity of the aqueous humour production, outflow and control. Here we should like to outline shortly the contemporary basic concepts of IOP regulation in respect to the possible role of steroid hormones. We feel that other factors cannot be neglected, however, we would like to put stress on steroids, especially on corticosteroids which are known to play the key role in the development of the iatrogenically induced intraocular hypertension, so-called "steroid glaucoma".

Aqueous humour formation

The site of aqueous humour formation is the ciliary body (Fig. 1). The current concept of this process was recently excellently reviewed by Cole [3, 4]. The vol. of the human eye chambre is approx. 300 μl, the flow rate under normal conditions reaches 3 μl/min. The production of aqueous humour appears to depend upon plasma osmolarity and on the rate of active solute transport by the epithelium. Ciliary epithelium consists of two cell layers lying apex to apex due to the ontogenic development of the eye. Facing the vessel-rich stroma of the ciliary body, the cells of pigmented epithelium are proximal, whilst the basal surface of the non-pigmented cell layers faces the posterior chambre (Fig. 2). All structural prerequisites for active transport mechanism are met in ciliary epithelium. The rate constant for entry of water from plasma to aqueous humour is almost ten times the corresponding values for solutes such as Na^+ or Cl^-. The blood-aqueous potential in rabbits is 5–7 mV,

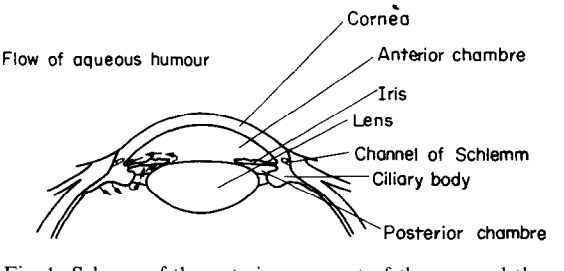

Fig. 1. Scheme of the anterior segment of the eye and the flow of aqueous humour.

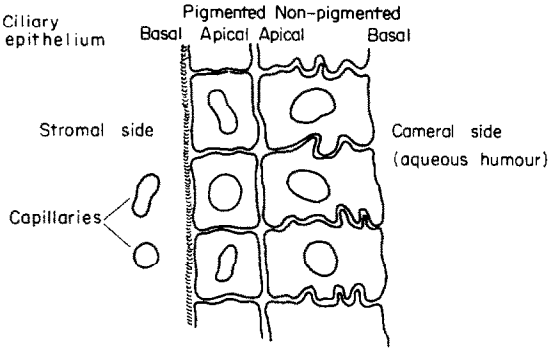

Fig. 2. Scheme of the two-layer ciliary epithelium.

the aqueous humour being positive to plasma. Na^+ is actively transported from plasma to the aqueous humour with the main anion, Cl^-, either migrating passively or being transported actively at a lesser rate. Transport is oxygen dependent and it is readily inhibited by cyanide and azide as well as by uncoupling agents and inhibitors of Na^+–K^+ ATP·ase, such as ouabain and ethacrynic acid. Non-pigmented cells are the main sites of enzyme activities as succinate dehydrogenase and ATPase, demonstrating the existence of a Na^+–K^+ATPase sodium pump energised by ATP. ATP is derived predominantly from oxidative metabolism of glucose *via* the citrate cycle. Diamond and Bossert [5] proposed a general model for standing gradient osmotic flow in which solute is actively transported into the lateral intercellular spaces between epithelial cells thus making channel fluid hypertonic. Cole [3, 4] applied this concept of standing gradient flow to the production of the aqueous humour. Junctional complexes localized at the apices of the non-pigmented cells act as a functional barrier. The lateral intercellular spaces of the non-pigmented epithelium are thus closed at the end nearer to the stroma and open at the cameral surface of the cells. Water enters the intercellular spaces across the cellular walls and, in the steady state, a standing osmotic gradient is maintained in the channel: tonicity falls during the passage from the transport site to the open end of the channel. The membrane ATPase represents secretory sites for sodium transport into the lateral intercellular spaces. An element of ultrafiltration could be superimposed on the standing gradient flow if "leaky" junction, demonstrable in the rabbit ciliary epithelium as well as in the small intestine, choroid plexus, and gall bladder or in proximal renal tubules, allows the possibility of some pressure dependent flow into the lateral intercellular channel.

Steroids and production of aqueous humour

Several similarities exist between the epithelium of the proximal renal tubule and that of the ciliary body. In contrast to the kidney which has been the subject of many physiological and biochemical steroid hormonal studies, there is as yet very little information on the action of steroid hormones on the rate of aqueous humour formation by the ciliary body. The known effect of glucocorticoids, which increase the IOP, and of oestrogens which occasionally reduce it, is rather due to a fall in the outflow facility than to a direct action on the ciliary epithelium, though this type of action cannot be excluded. There is some evidence that mineralocorticoids may increase IOP [6, 7] since blocking of endogenous aldosterone with spirolactone may decrease the rate of formation of the aqueous [8]. Recently it has been suggested that prolactin modulated the action of corticosteroids on ciliary epithelium so that a combination of prolactin and cortisol in the rabbit causes a marked increase of aqueous humour formation by acting on the epithelial transport system.

Steroids and outflow of aqueous humour

Following topical and sometimes also systemic application of glucocorticoids, a marked elevation of the IOP develops in some individuals. The sensitivity of IOP reaction to glucocorticosteroids has been shown to be genetically determined, most likely as an autosomal recessive trait. In some of the highly responding individual disbalance of the hypothalamohypophyseo-adrenocortical axis has been demonstrated, manifested by altered supressibility threshold of plasmatic cortisol after dexamethasone administration. The cases of so-called steroid glaucoma are rare but they can occur in patients using uncontrolled topical corticoid therapy for various chronic eye diseases. No definite conclusions on possible mechanisms leading to these pathological changes were drawn but unanimously they are searched and sometimes found [10] in obstructions in some structures of the trabecular meshwork in the angle of the anterior chambre. The obstructions decrease the outflow facility of the aqueous humour and result in an increase of IOP.

According to François and Victoria-Troncoso [11] the regulation of aqueous humour outflow is mediated by steady decomposition of mucopolysaccharides localized in the trabecular meshwork. Physiological steady state is achieved by the continuous depolymerization of these macromolecules by the action of catabolic enzymes liberated from lysosomes. Lysosomal membranes are stabilized by glucocorticosteroids which thus prevent the depolymerization of mucopolysaccharides and cause the obstruction of the main route of aqueous humour outflow. Theory of corticosteroid action *via* the stabilization of subcellular membranes is subjected to criticism. The effect on lysosomal membrane can be demonstrated only at extremely high concentrations of corticosteroids and it is questionable whether this mechanism of action could be applied for physiological or even normal pharmacological range of corticoid concentrations. For this and other reasons other authors see the cause of increased IOP in a disbalance of various metabolic factors caused by glucocorticoids [12, 13].

Biorhythm of IOP

In this connection it is useful to underline the fact that the course of the diurnal biorhythm of IOP curve [14] resembles in some respects that of glucocorticoids [15] and of aldosterone [16]. Other types of rhythmicity of IOP were observed in females. During the menstrual cycle the secretory phase is characterized by slightly higher IOP which decreases at ovulation and rises again in the luteal phase. The increase appears to be connected with estrogens while progesterone acts rather as a depressant of IOP [17]. Analogical effects of sex hormones were seen in pathologic climacteric [18]. Quite obscure is the mechanism of influence on IOP exerted by androgens: prolonged treatment with testosterone induced an experimental ocular hypertension in rats [19] and IOP was influenced by castration [20].

The action of steroid hormones

The present status of knowledge on IOP control is based mainly on clinical experience of physiological and pharmacological experiments. The biochemical approach has been rarely reported, though it might help to solve such problems as the divergent action of hormones on the formation and outflow of the aqueous humour, which could be supposed e.g. for mineralocorticoids.

We aimed to answer the following questions: Are the steroid hormones of endogenous origin available for eye tissues, especially those which are avascular and nourished solely by the aqueous humour as lens and cornea? How and to what extent are the steroid hormones metabolized in the eye and what biologically active metabolites are found? Does there exist a specific uptake and binding of steroid hormones which may start a sequence of events leading to biological effects? Some of these questions could be answered on the basis of our experiments on rabbit, human and bovine eyes.

Occurrence of steroid hormones in the aqueous humour

Isotope dilution, measurement of the radiochemical homogeneity, chromatographic properties and micro-

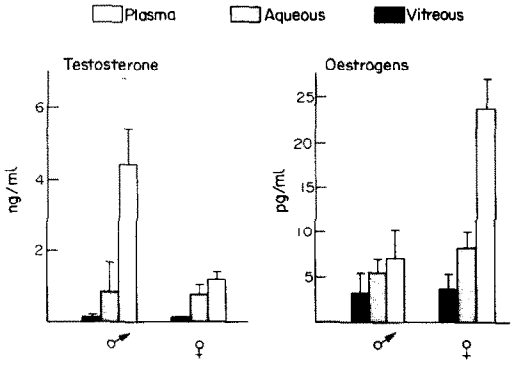

Fig. 3. Testosterone and oestrogen level measured by radioimmunoassay in plasma, aqueous humour and vitreous of male and female rabbits.

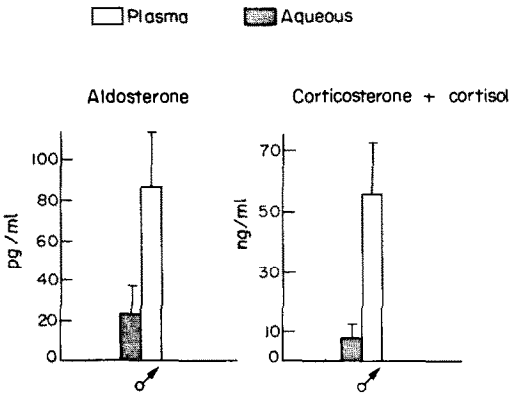

Fig. 4. Aldosterone and corticosterone (and cortisol) level in plasma and aqueous humour of the male rabbits.

reactions enabled the identification of corticosterone [22, 26], aldosterone [23], testosterone, oestrone and oestradiol [24] in the rabbit aqueous humour as well as cortisol in the human eye [25]. The levels of these hormones assayed by RIA [22–26] are lower than those in blood plasma (Fig. 3, Fig. 4) but higher than those corresponding to the concentration of free steroids in plasma. Cortisol, testosterone and oestrogens are not bound to any transport proteins in measurable amount in the aqueous humour.

Metabolism in eye tissues

The metabolism of steroid hormones under investigation in the eye tissues [22, 23, 27, 28] proceeds by the action of enzymes of steroid metabolism common in other steroid metabolizing tissues of the appropriate species (for rabbit see Table 1). In the vitreous and especially in the aqueous humour the steroid metabolism is slow if any. The differences in the intensity and direction of steroid hormones transformations between various tissues exist, e.g. in the cornea at pH 7.4, oxidation of 17β-hydroxygroup of testosterone and oestradiol-17β prevails in contrast to other tissues, but differences are rather quantitative. They may be important in the case of the formation of biologically active metabolites as dihydrotestosterone from testosterone which occurs markedly in the cornea, ciliary body, iris, retina and lens.

Binding of steroid hormones to receptors

The metabolic transformation of steroids may not have a direct connection to the biological action of the hormones. However, a biological activity could be anticipated for the hormones which show uptake and binding to specific tissue receptors. Until now such binding was found for aldosterone [23] in rabbit ciliary body but not in the cornea. On the contrary, testosterone was specifically bound to the bovine cornea. Aldosterone binding to the ciliary body tissue (Fig. 5) taken from non-adrenalectomized rabbits (because of troublesome adrenalectomy in this species) was determined at 0°C under 500 fold excess of dexamethasone and compared with the binding of

Table 1. Enzymes of steroid metabolism operating in the rabbit eye in *in vitro* conditions

Substrate	Eye tissue	Enzyme system
oestrone, oestradiol-17β	cornea, iris, retina, lens	hydroxylases 17β- and 17α-hydroxysteroid dehydrogenase
testosterone	cornea, iris, retina	hydroxylases 5α-reductase 3$\alpha(\beta)$-, 17α- and 17α-hydroxysteroid dehydrogenases
corticosterone, cortisol	cornea, iris, retina, lens	hydroxylases 3α-, 11β- and 20-hydroxysteroid dehydrogenases 5β- (and 5α-) reductase
aldosterone	cornea, iris	5β-reductase 3α-hydroxysteroid dehydrogenase

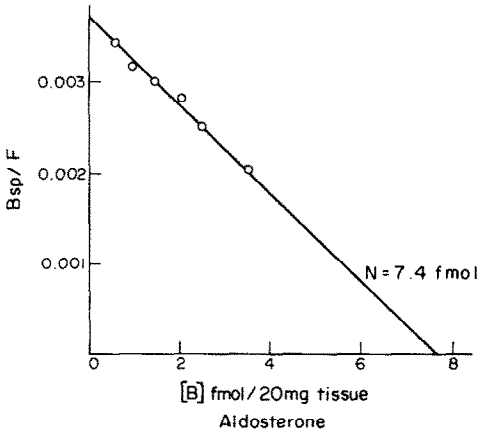

Fig. 5. Scatchard-like plot of aldosterone binding in ciliary body. Specifically bound aldosterone (B_{sp}) in 20 mg of wet tissue (2.56 mg of total protein) was calculated from the total bound radioactivity and corrected according to l.c. [30]. B_{sp}/F, where F = total unbound activity, was plotted versus B_{sp}.

cortisol. Cortisol binding under identical conditions is negligible and thus the binding of mineralocorticoid can be considered as specific with apparent association constant about 10^{10} mg/mol, the number of binding sites ranging from 0.20 to 0.40 fmol/mg of wet tissue in the situation where a part of binding sites are occupied by endogenous aldosterone. This low but definite binding corresponds to the suspected role of aldosterone in the control of sodium transport in the ciliary body. The findings are in agreement with our observations of absent action of aldosterone *in vivo* and *in vitro* as well as that of spirolactone *in vitro* on the transfer of ^{22}NaCl through the cornea [29] and measurable though small effect on ^{24}NaCl transport from blood to the aqueous humour through the ciliary body to the aqueous humour.

CONCLUSION

The IOP regulation should be investigated more in detail from the point of view of steroid hormone action. The first steps of the approach described here seem to promise that in the future, the biochemistry of steroids, the study of their interactions with proteins and their anticipated role in ocular transport phenomena, may contribute to understanding the basic pathophysiological processes underlying glaucoma.

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