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A major pathway for the regulation of intraocular pressure

Marvin Sears & Alden Mead
New Haven, U.S.A.

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Abstract

There has been a suspicion on the part of many clinicians and research scientists that intraocular pressure can be regulated by neural and/or humoral influences upon the rate of aqueous humor formation. It has been difficult, if not impossible, to separate specific influences of the central nervous system upon intraocular pressure from vascular induced or other secondary alterations. The past two decades have witnessed a great deal of study of the role of the adrenergic nervous system upon the regulation of intraocular pressure. From the investigations it is possible to formulate an integrated concept that can place years of work and speculation on a firm molecular foundation. The secretory tissue of the eye, the ciliary processes, contain an enzyme receptor complex, comprised by receptor complex, comprised by receptor bound membrane proteins, the catalytic moiety of the enzyme, a guanyl nucleotide regulatory protein (or N protein) and other features. The enzyme can be activated by well known neurohumoral or humoral agents that consist of catecholamines, glycoprotein hormones produced by the hypothalmic pituitary axis, and other related compounds, including placental gonadotropin. These compounds cause the ciliary epithelia to produce cyclic AMP at an accelerated rate. Cyclic AMP, as a second messenger, causes, either directly or indirectly, a decrease in the rate of aqueous humor formation that may be modulated by cofactors. Clinical syndromes fit the experimental data so that an integrated explanation can be given for the reduced intraocular pressure witnessed under certain central nervous system and adrenergic influences. The molecular biology of this concept provides important leads for future investigations that bear directly both upon the regulation of intraocular pressure and upon glaucoma.

Introduction

The purpose of this review is to assemble old and new observations of the effects of the central nervous system and the adrenergic nervous system upon intraocular pressure (IOP). It is now possible to unify our concept of the ocular pressure effects of these systems because physiologic studies have indicated a very central role for the adenyl cyclase receptor complex as a regulator of aqueous humor formation (16, 46, 47). Evidence for the regulatory role of ocular adenyl cyclase based on results from studies of the adrenergic nervous system can be integrated with results from studies of the neurohumoral influence of the central nervous system upon IOP to develop a unifying hypothesis telling how these two systems, central nervous system and adrenergic system, may exert their effects upon aqueous humor dynamics.

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Methods

Interest in the adrenergic control of the resistance to aqueous outflow was rekindled when it was found that the 24 hour decrease in IOP after postganglionic adrenergic denervation could largely be accounted for by a decrease in the resistance to outflow of aqueous humor (23, 44). Other laboratories joined the race to learn about adrenergic influences on outflow (11, 37). The chase became even more spirited when circumstantial evidence was presented that linked the production of cyclic AMP and its persistence in the anterior chamber aqueous humor with decreased IOP and increased outflow of aqueous humor (30). It has become clear, however, that, even in the laboratory, only large doses of adrenergic drugs and some of their analogs can induce the effect, and, that even this pharmacologic effect, while definite, is small (32, 33). With the appearance of utilizable techniques for measurements of aqueous inflow, attention now shifted to include these latter data (1, 7, 21). Again, however, adrenergic effects are small and varied, have not been characterized by study of dose dependency, are related to routes of delivery of the drug wherein frequently the delivery cannot be quantified, and exhibit species differences that in fact may not be as related to the species as to the experimental method. Finally, conclusions drawn about the mechanism of adrenergic effects do not always reflect a convincing application of known cellular receptor mechanisms to explain the clinical effect of these drugs (34). In spite of these discrepancies, study of the adrenergic system has proved to be a stimulating way to learn about regulatory mechanisms for IOP. In particular, it has led us to the development of information about receptors on a molecular level. An enzyme-receptor complex is present in the tissue responsible for the formation of aqueous humor. The adenyl cyclase receptor compex is present in the secretory epithelia of the ciliary processes of the eye and is associated with the other components of the second messenger system (45). If adenyl cyclase were linked to a common receptor or even to different receptors activated separately by either a central mediator or an adrenergic one, it would provide an integrated explanation of the action of these systems.

A physiologic technique useful for the study of the receptor-containing tissue responsive to central and adrenergic mediators is one of the requirements for an adequate investigation. A probe of this receptor must have these characteristics: 1. potency; that is, activity at physiologic or low concentrations. Some agents delivered by intraocular perfusion or by topical application can exert effects on the outflow channels and related structures because they reach these targets in concentrations much larger than would be anticipated if similar compounds were regularly gaining entry to the eye by customary blood routes after endogenous release. Blood levels of hormones, for example, are in the range of 10^{-10}M, but in pharmacologic studies, of the eye done with adrenergic agents, for example, levels of hormones in the aqueous humor three to five orders of magnitude greater are required even for modest effects. 2. Effectiveness in the undisturbed eye to avoid possible changes induced by disruption of the blood-aqueous barrier. 3. A prolonged duration of action to avoid any secondary effects produced by short lived vascular changes. A good candidate satisfying these three requirements was the cholera toxin. Cholera toxin has been widely used as a classic stimulator to evaluate the function of adenyl cyclase in various tissues. The prolonged action of the cholera toxin is a particular property that makes it ideal for intraocular study of the dynamics of aqueous humor. Changes from the toxin will become manifest after an interval of several hours. At this later interval, any earlier changes in blood flow can be separated from the slower changes in either aqueous humor formation or in outflow resistance that may have occurred to create a new steady state IOP.

A test of this idea was executed in the summer of 1977 when Marion Stoj, a medical student, and Alden Mead studied the effect of cholera toxin on IOP in our laboratory (47). Intravitreal injections of cholera toxin were made into the eyes of rabbits and the IOP fell dramatically to levels of episcleral venous pressure (16). Of course, in large doses, 2 mg or more per vitreous cavity, the ocular tissues showed inflammatory changes. Of enormous importance, however, was the fact that IOP could be reduced with much lower doses without any trace of inflammation. As little as 0.015 mg (10^{-11}M final

dose in vitreous cavity) still produced a dramatic effect (46). The interval required for the response varied slightly, but within 12 hr virtually all animals exhibited at least a 5 mm Hg drop in IOP.

Encouraged by the results obtained from intravitreal injection, experiments were done in which cholera toxin was administered by close arterial infusion but accomplished so that the ocular circulation was not compromised. Delivery of cholera toxin through the blood supply was particularly important because under ordinary conditions it would be expected that hypothetical endogenous mediators would arrive at the ciliary processes in this manner. It turned out that arterial infusions of cholera toxin caused a marked decrease in intraocular pressure. The effects were so remarkable that we decided to study in detail the relationship between the rate of aqueous inflow, blood flow, and the reduction in intraocular pressure. After preliminary dose-response experiments it was decided to infuse rabbits with 2.4×10^{-11} M of cholera toxin through the right internal maxillary artery. IOP and blood flow are shown in Fig. 1. In the phase before (and during) the reduction in IOP, cholera toxin actually increased the blood supply to the anterior uvea. This means that the decrease in

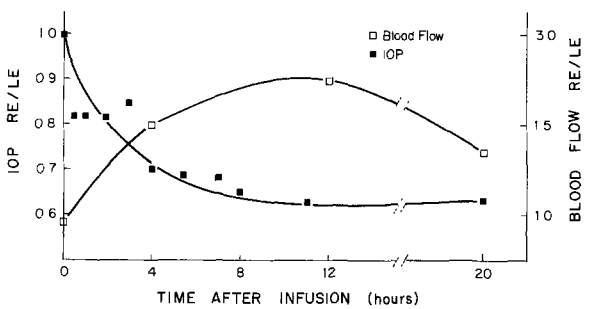

Fig. 1. Effect of close right arterial infusion of cholera toxin on ocular blood flow and IOP. IOP and blood flow are plotted as ratios R/L, the RE was experimental and the LE control. Blood flow values at each interval represent a mean of values from four rabbits taken immediately after infusion, and 4, 12 and 20 hours later. Ten additional animals had measurements of IOP by applanation tonometry at the intervals shown. IOP decreases as blood flow increases. Early increases in blood flow, within the first few hours, are an effect of the cholera toxin. Later increases may include an indirect effect or component of hypotony to increase blood flow further. (From M.L. Sears et al., reference 47).

aqueous flow cannot be caused by limited blood flow to the ciliary processes. Measurements of aqueous flow revealed that cholera toxin in eyes of rabbits whose blood ocular barrier were intact reduced the formation of aqueous humor by more than 50% from a normal value of 2.4 ± 0.09 (12) to 1.1 ± 0.15 (6) μl/min $p < 0.01$. The decrease in IOP induced by arterial toxin was thus caused by drastically reduced aqueous flow.

The anatomic localization of the binding sites for the action of cholera toxin in the eye has been studied (27). Cholera toxin reduces the rate of formation of aqueous humor in concentrations (10^{-11}M) that do not disturb the morphology of the aqueous humor forming epithelial cells of the ciliary processes of the rabbit eye and that do not disturb the blood aqueous barrier. Furthermore, cell surface reception which mediates the action of cholera toxin on aqueous humor formation is localized in the interdigitating and apical plasma membranes of the nonpigmented epithelium (Figs. 2 and 3). Thus cholera toxin delivered by close arterial infusion (or by intravitreal injection) causes a profound reduction in IOP by decreasing net of aqueous flow through the eye without compromise to the aqueous forming cells or to their blood supply. These results are consistent with an influential role for the adenyl receptor cyclase complex of the ciliary process epithelia as a determinant of levels of IOP. Cholera toxin activates adenyl cyclase in the ciliary processes of rabbit eyes (16, 47) and can also increase the rate of production of cyclic AMP in intact ciliary processes from human eyes. In these human eyes, several of them quite fresh, basal cyclic AMP levels in excised ciliary processes averaged 5.2 picomoles/mg of cell protein and could be raised almost tenfold by stimulation with cholera toxin (47). It turns out that in addition to cholera toxin, beta adrenergic agonists and glycopeptide hormone preparations can increase the rate of production of cyclic AMP by the ciliary processes, to reduce net aqueous flow and to cause a reduction in IOP (47).

Are there any other endogenously released substances that can mediate changes in aqueous flow in a similar manner? The answer to this question could tell us whether the mechanism by means of which

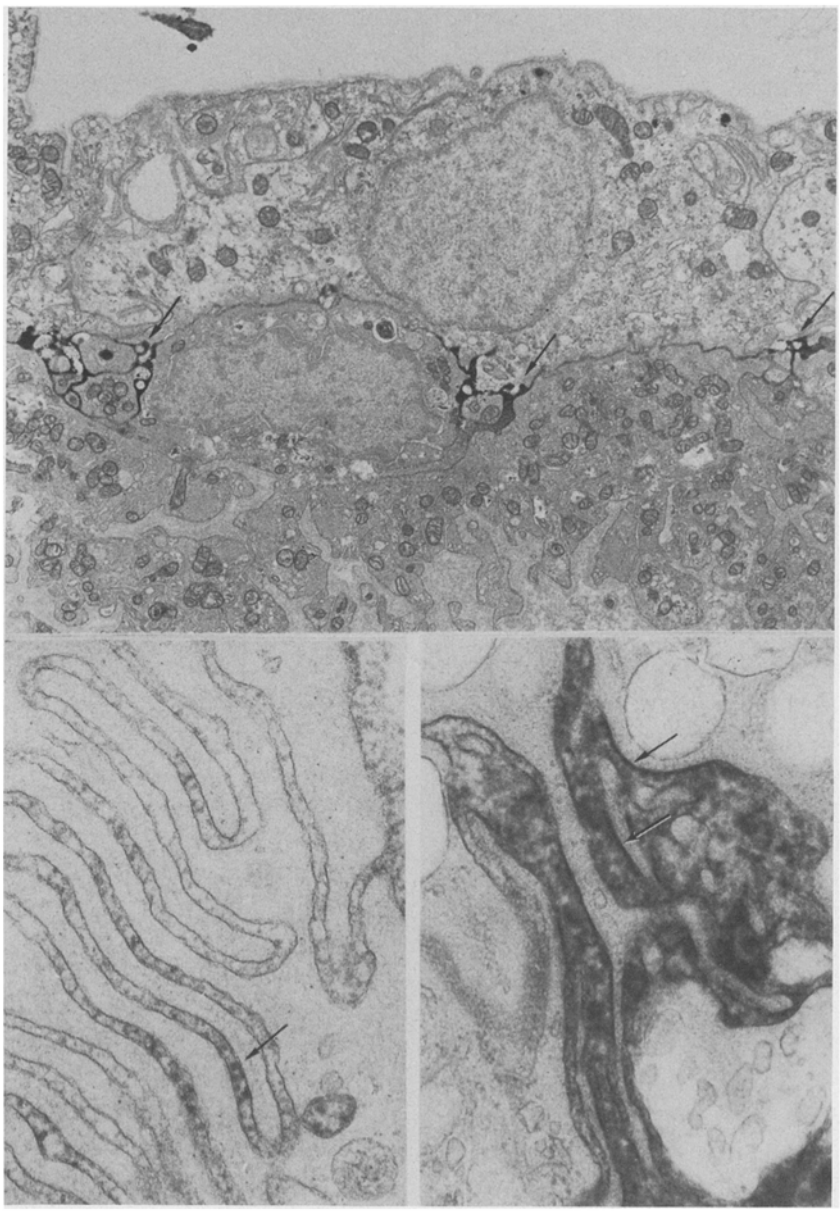

Fig. 2. Top: Electron micrograph of part of a ciliary process taken after close arterial infusion with B subunit of cholera toxin conjugated with horseradish peroxidase. Reaction products (arrows) within apical extracellular spaces between PE and NPE localize binding sites of cholera toxin. × 6000

Bottom left: Lateral interdigitating membranes of NPE cells show beaded reaction products on outer surface of plasma membrane. X 60,000

Bottom right: Extracellular space between PE and NPE. Reaction products attached to PE or NPE plasma membranes show binding sites of cholera toxin. × 45,000 (modified from Mishima et al., reference 27).

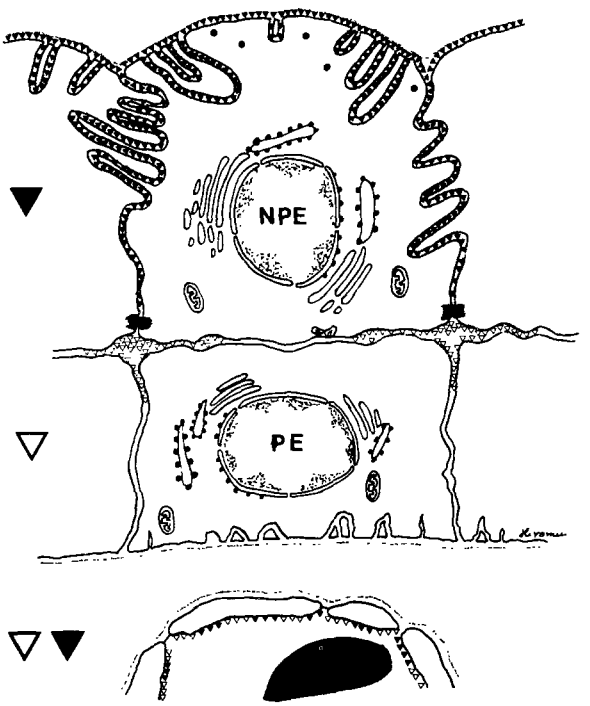

Fig. 3. Summary diagram of distribution of binding sites on ciliary processes of beta subunit of cholera toxin and likely sites for beta subunits or glycopeptide hormones: ▼ = after in vitro incubation; ▽ = after close arterial delivery.

influences of the central nervous system affect IOP can be correlated with the effects of the adrenergic nervous system on IOP. Before we address this latter question, a short digression to discuss another aspect of adrenergic influences on IOP is indicated. Although epinephrine, a mixed alpha and beta adrenergic agonist, was first used topically in the 1920's to treat glaucoma (17), it was Weekers (54) who first demonstrated that isoproterenol, a beta adrenergic agonist, reduced pressure in the human eye. Later, Ross & Drance (41) showed the dose response relationship between isoproterenol and reduced IOP in humans. The dramatic reduction in IOP occurred without affecting gross facility of aqueous outflow. It was therefore concluded that isoproterenol lowers IOP by reducing aqueous humor formation. Gaasterland (13) et al. proposed that beta adrenergic stimulation done by topical isoproterenol caused a 60% reduction of aqueous humor inflow in four young normal males. In the animal laboratory, Eakins (10), using the technique of intravitreal injections, studied aqueous humor outflow directly and aqueous humor formation indirectly under the influence of epinephrine, norepinephrine, isoproterenol. While systematic errors may have occurred, a clear-cut separation of drug effects was noted, with isoproterenol, the classic beta agonist, reducing aqueous humor formation. All these cited studies indicate very clearly that stimulation of the beta adrenergic receptor, adenyl cyclase, lowers IOP, probably by reducing aqueous humor formation.

The demonstration in the 1960's and the years to follow, that several beta adrenergic blockers (4, 38, 44), but especially timolol (50), lower intraocular pressure, and, that the latter does so by a reduction in aqueous humor formation (57, 58), has provided a good deal of excitement for both clinicians and researchers. For the clinician a very potent useful topical agent has been developed in the treatment of glaucoma. For the researcher, the task of unraveling the apparent discrepancy of adrenergic agonists and antagonists acting in the same direction has proved interesting. Stimulation of adenyl cyclase reduces net aqueous flow. How then can blockade of adrenergic receptors lower intraocular pressure by reducing aqueous flow? Either ocular receptors are different from the classic beta adrenergic receptors or timolol acts in a unique manner unrelated to its action as a beta adrenergic blocker. Evidence for beta adrenergic activity (31, 53), and receptors in the ciliary processes (6, 15) has been collected and further characterization indicates that very likely $beta_2$ adrenergic receptors are present (29). These are, by definition, essentially the same receptors as found in vessels that mediate vasodilation and in the lung that mediate bronchodilation. Therefore the possibility that ocular beta adrenergic receptors are different from the classic beta receptors is remote. In defense of the position that beta adrenergic blockers work as such to lower aqueous humor formation it is said that the beta blockers as a class of drugs lower IOP. Timolol is simply the most efficacious. Nonetheless, there is not apparent parallelism between the ability of a drug to reduce IOP and to block beta receptors. Second, timolol acts like a beta adrenergic blocker in vivo at a concentration lower than that required for its pressure reducing effect in the eye and yet at a

concentration consistent with its Kd for binding to beta adrenergic receptors in ciliary processes (15). Third, d-timolol lowers IOP (24). This finding indicates a lack of stereospecificity for the beta receptor. Fourth, the suggestion that timolol or other beta blockers may have a presynaptic locus of action does not help because it has been found that the drug is equally effective in eyes that are normally innervated and postganglionically denervated (55). Although the authors themselves did not draw this conclusion with respect to the locus of action of timolol, it is quite clear that in the presence of a postganglionic denervation, a presynaptic locus of action with timolol would be eliminated. A speculation has been made that timolol or beta blockers may inhibit ciliary process Na-K-ATPase[2] but the doses required for this in vitro effect are very much larger than required for the drug to exhibit other chemical, physiologic, or pharmacologic effects. For all these reasons, it is likely that timolol reduces aqueous formation by a mechanism in addition to or other than its beta blocking action. This conclusion is an explanation consistent with the powerful evidence that stimulation, not blockade, of adenyl cyclase reduces aqueous flow (10, 13, 16, 41, 46, 47, 54).

Now we can turn our attention to the central nervous system. If the adenylate cyclase receptor complex is regulatory for aqueous humor formation, are there other agents besides adrenergic compounds and cholera toxin that can stimulate this complex to reduce aqueous humor inflow? It turns out that preparations of important hormones elaborated by the hypothalmic pituitary axis of the central nervous system have the ability to stimulate the adenyl cyclase-receptor complex and can lower IOP and aqueous humor formation. These are preparations of the glycoprotein hormones, the gonadotropins.

Central neural and/or neuro-humoral influences upon IOP were the subject of speculation and have been searched for many years. In 1870, Von Hippel & Gruenhagen (51) speculated that the cervical sympathetic nerves decreased and the fifth nerve increased pressure. Elwyn (12) in 1938, Hess (18) in 1945, Magitot (25) in 1947, Schmerl & Steinberg (43) in 1950 and Duke-Elder (9) in 1957 all hypothesized that a center in the hypothalmus or diencephalon was responsible for regulation of eye pressure. Von Sallman & Lowenstein (52) tried to explore the diencephalon to find specific areas that could alter IOP independently of changes in systemic blood pressure. The changes in eye pressure that could be recorded were only transient. In 1951, Nagai, Ban & Koratsu (28) showed changes in IOP after stimulation of the diencephalon but believed that these changes were not independent of alterations in systemic blood pressure. These neurophysiologic studies on the central nervous system have not delineated a pathway that can mediate IOP. More recently, a relationship between changes in blood osmolality and IOP have suggested that the hypothalmus mediates these fluctuations in IOP by way of efferent fibres in the optic nerve (22, 40). Additional supporting evidence is required to establish a definite pathway and its effector.

Clinical studies have revealed two endocrine instances in which hypotony or low IOP occurs without ocular pathology. One of these is pregnancy. The drop in eye pressure in pregnancy has been studied sporadically over the last twenty years. Niebroj (36) in 1971 showed that administration of chorionic gonadotropin in menopausal patients with glaucoma in a dose of 10,000 units daily over a period of five days caused a slight to moderate drop in pressure after treatment. A previous study by Niebroj (35) had shown that in certain glaucoma patients there were decreased LH levels. There had been some other earlier studies of patients who were pregnant. For example, DeGrosz (8) in 1937 showed that a series of pregnant women had a 2 to 3 mm drop sustained throughout their pregnancy. In Scandinavia, Wilke (56) showed a 1.4 mm Hg fall in IOP only during pregnancy and believed that this pressure drop could be accounted for by a change in episcleral pressure. Horven (19) in 1974, in a work designed to study the corneal indentation pulse in pregnant women, measured IOPs and found these to fall from an average of about 14 mm Hg to a low of 11 to 12 mm Hg in the 30 to 40th week of pregnancy. No mention was made of any relationship to circulating hormones. Seven other studies referenced in Horven's paper were quoted as agreeing that IOP decreases in pregnancy. Among

these studies, an early one by Becker & Friedenwald (3), two women were found to have increased outflow. The authors commented that the increase in outflow was related to an increased circulating level of progesterone. Horven himself comments on the possible relationship of IOP to episcleral venous pressure as a determining factor but makes no mention of possible changes in aqueous humor formation. He did observe, however, that the IOP goes down in the first trimester and apparently stays down. In perhaps one of the first studies of pregnant women, Imre (20) in Hungary, showed that 42 of 50 pregnant women studied had tensions of 12 mm Hg which he characterized as being much less than the 20 mm Hg which he considered normal.

The other clinical condition in which hypotony has been documented (and in which gonadotropins (FSH) have been shown to be elevated) is myotonic dystrophy. The authors who originally described the ocular findings in myotonic dystrophy (5) and others who have confirmed the observation of hypotony did not make a connection to any particular humor to account for the drop in pressure. It should be remembered that male patients with myotonic dystrophy have testicular gonadal atrophy. Probably as a consequence increased circulating levels of FSH have been noted. In pregnancy increased chorionic gonadotropins (of placental origins) and in myotonic dystrophy increased FSH levels occur. These changes can well account for the drop in IOP seen in these two groups of patients. As one can also conclude from the ensuing experimental studies, there is a correlation between the elevated glycoprotein hormone levels and reduced IOP. Further study of the effect of these glycoprotein hormones obviously required this scrutiny in a controlled environment.

Glycoprotein hormones include the pituitary and the placental gonadotropins and the thyroid stimulating hormone of the pituitary. These hormones are very complex proteins that have diverse functions, yet are closely related in structure. Each bears two peptide chains or sub-units, alpha and beta. They are cross linked by disulfide bonds and are glycosylated at specific residues. Specificity of hormonal activity, however, is conferred by the beta sub-units. The alpha sequence is virtually identical for all the glycoproteins. These hormones elicit their biologic response after interaction with cell surface receptors by activation of adenyl cyclase and the production of cyclic AMP (42). The results with the cholera toxin, the classic stimulator of the adenyl cyclase receptor complex, and with catecholamines. indicating that stimulation of beta adrenergic receptors causes a net reduction in aqueous humor flow (16, 46, 47), leads to an obvious explanation of the mechanism of action for the hormones of the adrenergic system on the one hand and the cholera toxin and glycoprotein hormones on the other. All of these chemicals act on a cell surface receptor linked to stimulate membrane bound adenyl cyclase to reduce net aqueous humor flow (42, 47). New evidence from experiments done with gonadotropin preparations supports this hypothesis.

We obtained and then tested two different preparations of each of the gonadotropins. These preparations with their bioassayed activity according to the 2nd international standard were: bovine thyrotropin (TSH) from Armour, (Thyrotropar) and bovine TSH a gift from Professor John Pierce of UCLA, about 10 μ/mg; follicle stimulating hormone (FSH) from Sigma Chemical Corp. (50 μ/mg) and from the National Institutes of Health (Lot #1366) 60 μ/mg; luteinizing hormone (LH) from Sigma (about 6,000 μ/mg); (HCG) human chorionic gonadotropin from Serono (about 6,700 μ/mg) and Pregnyl from Organon (about 2,500 μ/mg). These preparations were studied in rabbits by administering a right intravitreal injection of a test substance in 10 μl of diluent while the left control eyes received a 10 μl injection of the diluent itself. Additional controls included two different progesterone preparations and quingestanol, generously donated by Dr. Black of Warner-Lambert. A typical experiment done with two different doses of an HCG preparation, as an example, is shown in Fig. 4. About 8 hours after intravitreal injection eye pressure begins to fall and reaches its ebb within 16 hours. The effect may last as long as two days. The time course of this local ocular effect, relatively slow onset and prolonged duration, is quite consistent with the way in which these compounds act on the gonads. Both these effects are of course

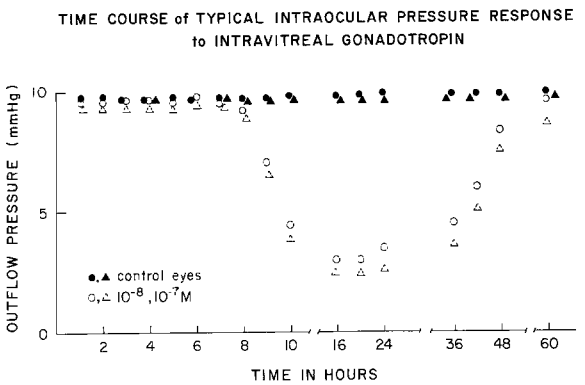

Fig. 4. Human chorionic gonadotropin (pregnyl, Organon) was injected intravitreally into the right eyes of rabbits in a volume of 10 μl to produce final molar concentrations of 10^{-8} M in one series and 10^{-7} M in a second series. The control left eyes were injected with 10 μl of diluent. Outflow pressure (applanation pressure) minus episcleral venous pressure (unchanged usually at between 8–10 mm Hg) is plotted on the ordinate. After six to eight hours IOP begins to fall and reaches a nadir at about 16 hours. The reduction in pressure persists for almost two days before returning to normal control levels.

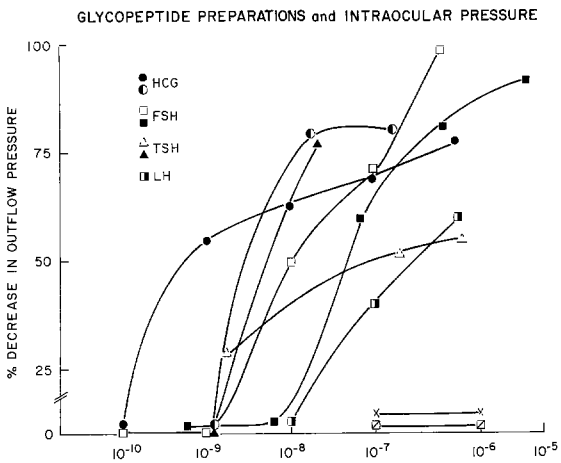

Fig. 5. A summary dose response curve showing the effect of several commercial glycopeptide preparations upon ocular outflow pressure in the rabbit reflecting the time of peak response, usually about 16 hours. Each point represents the mean of 5 to 8 eyes. ×, progesterone; □ quingestanol, given to animals followed for 24 hours, had no effect in doses plotted.

based upon activation of cell surface receptors that are coupled with adenylate cyclase to cause an accelerated rate of production of cyclic AMP (42, 47). The physiology of this response is virtually the same as that of cholera toxin: a pronounced action of these compounds to reduce aqueous humor formation accounts for the reduction in intraocular pressure. The results obtained with all the gonadotropin preparations plotted at the time at which their maximum effect was accomplished, about 10–14 hours after intravitreal injection, are illustrated in Fig. 5. At levels of from 10^{-10} M to 10^{-6} M, final vitreal concentration, dramatic effects in lowering intraocular pressure were obtained for each of the compounds tested. FSH and HCG were more potent, followed by TSH. LH was the least effective with the preparations and dose levels used. Not surprisingly, progesterone and its congeners were completely ineffective. These latter compounds interestingly inhibit activation of adenylate cyclase (12).

It is interesting simply to compare the final molar vitreal concentration required to give a consistent pressure reducing effect with the levels of human gonadotropin found in the plasma of pregnant women during the first trimester. These plasma levels for HCG are 10^{-7} M tested by radioimmunoassay, a figure that in the vitreous consistently produced a significant fall in IOP (Figs. 4 and 5). Could then this local effect be reproduced by parenteral administration? Males, females, and oophorectomized female rabbits were given pergonal in a dose of 25 units of FSH and 25 units of LH intramuscularly daily for three doses while control animals were given a diluent. Six days after the first injection the procedure was reversed; animals that received diluent were now given injections of pergonal and the hormone injected animals were given diluent. Fig. 6 indicates the results. The males, females and oophorectomized animals are the three groups traced respectively (top to bottom) in the figure. Parenteral administration of the FSH-LH preparation produced an effect on intraocular pressure which began within 24 hours and lasted for 2 to 3 days in all groups. Although three doses were used it is clear that the first dose alone could bring the pressure down. This result in animals again confirmed the impression encountered in pregnant human females and in myotonic dystrophy patients that elevated levels of gonadotropic hormones can reduce intraocular pressure. The effect is clearly not mediated by the gonads because of the equivalent

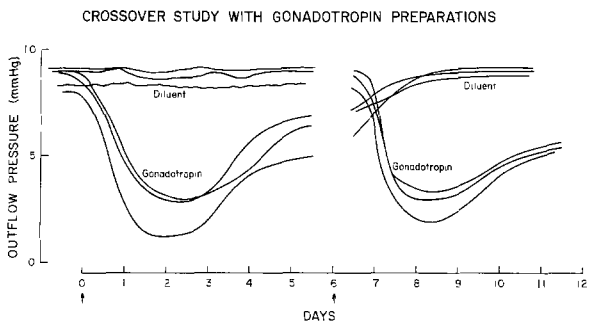

Fig. 6. Tracing indicates the response of outflow pressure to parenteral injections of FSH 25 μ and LH 25 μ administered together in a preparation called pergonal (Serono). 6 males, 6 females, and 6 oophorectomized females, (respectively top to bottom traces) were divided into two groups that reversed 6 days later. A dramatic response began after the first of three injections to reduce outflow pressure to 75% or more (in the oophorectomized group). Crossover study confirmed the result, indicating that parenteral gonadotropins can reduce intraocular pressure significantly.

effects in three groups. In fact, the oophorectomized group had the greatest effect. Also, the gonadotropin effect can be established from either the blood or vitreal side of the ciliary epithelium.

Although our measurements of net aqueous flow (16) after activation of ciliary epithelial adenylate cyclase cannot distinguish enhanced aqueous reabsorption from reduced secretion, a schema of how several compounds may act on the adenylate cyclase receptor complex in the ciliary epithelium is indicated by Fig. 7 and its legend. Fig. 8 summarizes different influences on the adenylate cyclase receptor complex.

Additional experiments are needed both on clinical and experimental levels to support the hypothesis proposed in this paper. It could turn out, for example, that the preparations used in the current experiments reported contain an impurity or a new substance with pressure reducing activity. Nonetheless, it is quite clear from these laboratory experiments and may be surmised from other clinical studies that the net flow of aqueous humor can be dramatically reduced by activation of the adenylate cyclase system. The decrease in net aqueous flow is associated with the activation of adenylate cyclase contained within the ciliary processes (of the eyes of both rabbits and humans). Preparations of glycopeptide hormones whose action is mediated by the adenylate cyclase system also lower

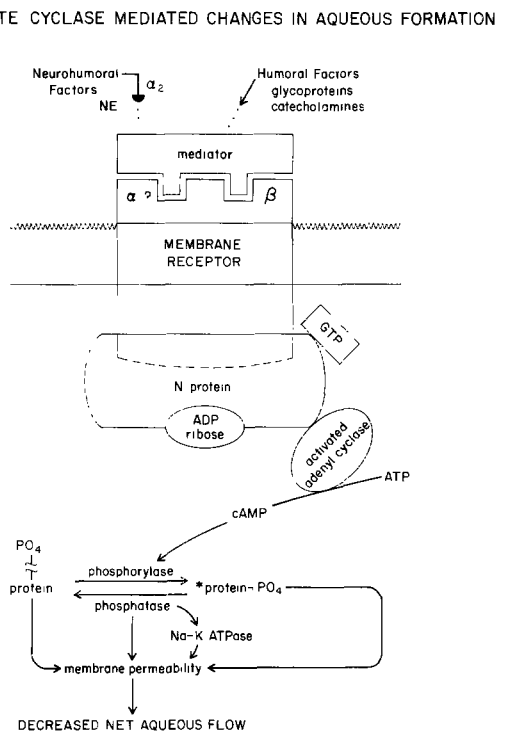

Fig. 7. When mediators act on a beta membrane-bound receptor, the catalytic moiety of the adenylate cyclase complex, is activated via the coupling protein, N, that binds GTP. It is possible, similar to the exogenous ribosylating action of cholera toxin, that endogenous ADP ribosylation of the N protein occurs within the cytoplasm of the cell. In this ribosylated state of the N coupling protein, an associated GTPase is inhibited, an effect that keeps adenyl cyclase activated. (The regulatory role of the guanine nucleotide may include an amplifying effect by GTP and a dampening effect by GDP.) Cyclic AMP is now produced at an accelerated rate and activates or deactivates a phosphorylation system which may directly regulate membrane permeability or may indirectly regulate the rate of formation of aqueous humor by altering the rate at which sodium is presented to an Na-K-ATPase pump.

In ciliary process the beta receptor may be a $beta_2$ receptor rather than both beta 1 and 2. Whether these receptors are distributed evenly or differently across the epithelia and vasculature is not known at this time.

Besides beta receptors, there are alpha (α) receptors linked to inhibit adenyl cyclase. These have not yet been demonstrated for ocular tissue. (They are therefore shown with?) There may occur other alpha (α) receptors, $alpha_1$, that are post-synaptic, and not linked to adenyl cyclase. These may influence the ciliary process through calcium as a second messenger to produce either vascular or, less likely, epithelial (secretory) effects. These have been demonstrated in the lacrimal and parotid gland, but not for ciliary process. Finally, there are presynaptic, $alpha_2$ receptors that act in feedback, to inhibit norepinephrine synthesis. The effect of these receptors in the ciliary process is unknown at present but they are known to function elsewhere, as in the retina.

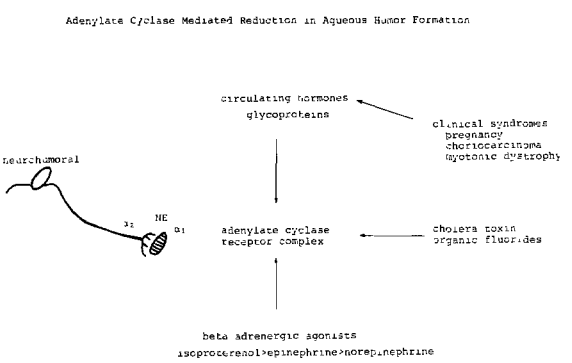

Fig. 8. A simple summary diagram indicates how neurohumoral changes may be mediated by norepinephrine (NE) at a postsynaptic noncyclase receptor, α_1, or by catecholamines at the beta receptor, and other hormones at another membrane bound receptor, coupled to act upon the catalytic moiety of the receptor enzyme complex, adenylate cyclase.

intraocular pressure. These effects are not mediated by estrogen or progesterone (Fig. 5). [The latter have small effects on IOP (26, 48)]. Thus experimental results from studies of the adrenergic nervous system and from the central nervous system as well as clinical observations (5, 8, 14, 20, 35, 36, 39, 47, 48) dealing with hormone preparations that are elaborated by the placenta or after release from the hypothalamic pituitary axis of the central nervous system have increased our understanding of how IOP may be regulated through changes in aqueous humor formation. The biology of the ciliary epithelium tells us that the membranes of these cells contain a common receptor coupled to adenylate cyclase. The kinetics of its occupancy by agonists and antagonists have been and are under intense study (6, 15, 29, 31). Regulation of this enzyme receptor complex can take place by events outside the cell, i.e. neural or humoral ones, at the cell membrane receptor, or by events at the level of the coupling mechanism within the cell membrane, or by intracellular components dampening or enhancing the activation of the catalytic moiety of adenylate cyclase (47) (see Fig. 7). Although the precise schedule of molecular events that occur after interaction of a hormone with its membrane receptor are complex, future studies of the formation of aqueous humor will undoubtedly be dependent upon and reflect a chemical dissection of these loci.

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Authors' address:
Department of Ophthalmology and Visual Science
Yale University School of Medicine
310 Cedar Street
P.O. Box 3333
New Haven CT 06510
U.S.A.