Forskolin is a diterpene derivative of the plant Coleus forskohlii that stimulates adenylate cyclase activity without interacting with cell surface receptors. Forskolin lowers the intraocular pressure of rabbits, monkeys, and humans. In rabbits, net aqueous humor inflow decreases, outflow facility remains unchanged, and ciliary blood flow increases. Tolerance to the intraocular pressure lowering effect did not occur in rabbits after topical doses given every 6 hr for 15 days. In vitro forskolin activates adenylate cyclase of crude particulate homogenates prepared from cultured human ciliary epithelia or from dissected ciliary epithelial processes of rabbit or human eyes. This activation is not blocked by timolol. The stimulation of adenylate cyclase by isoproterenol in vitro is potentiated in the presence of forskolin. Forskolin represents a potentially useful class of antiglaucoma agents differing in molecular mechanism of action from previously used drugs. Invest Ophthalmol Vis Sci 25:268-277, 1984

The stimulation of adenylate cyclase in the ciliary epithelium by cholera toxin,2-3 by commercial preparations of gonadotropins,4 or by activation of beta receptors5 can lower intraocular pressure (IOP) by reducing net aqueous inflow.6 Thus, receptors that are coupled functionally to ciliary epithelial adenylate cyclase can be activated by beta adrenergic agonists, such as isoproterenol, and certain gonadotropic hormones (especially FSH and hCG).4 Forskolin is a unique diterpene derivative of the plant Coleus forskohlii that acts independently of cell surface receptors to increase intracellular levels of cyclic AMP (cAMP).10-15 It has been used experimentally for its positive inotropic and blood pressure lowering effect. We have demonstrated recently that topical forskolin significantly reduces IOP in rabbits, monkeys, and humans.16

The purpose of this follow-up work was to examine in further detail the effect of forskolin on the eye by studying (1) the effect of intravitreal forskolin on rabbit IOP; (2) the detailed dose–response relationship of topical forskolin in rabbits; (3) the potential cardiovascular side-effects of topical forskolin; (4) the systemic and ocular effects of forskolin after intravenous administration; (5) the effects of topical forskolin on regional ocular blood flow; (6) the effect on aqueous flow by topical forskolin using a new method of intravitreal injection of fluorescein–dextran17; and (7) the in vitro effects of forskolin on human and rabbit ciliary adenylate cyclase activity.

Materials and Methods

Forskolin was obtained from Calbiochem-Behring Corporation (La Jolla, CA), 1-isoproterenol and fluorescein–dextran from Sigma Chemical Company (St. Louis, MO), and timolol was donated by Merck, Sharp & Dohme (West Point, PA). Radioactive microspheres were obtained from New England Nuclear Corporation (Boston, MA).

Animals

Male New Zealand white rabbits weighing 2.0–2.5 kg were used. Monkeys were Macaca arctoides weighing 10–15 kg.

Subjects

Human subjects were normal male and female volunteers with no history of eye disease and normal eye examinations. Informed consent was obtained from each subject.

IOP Measurements

Rabbit IOP was measured after a drop of topical proparacaine using an applanation pneumotonometer calibrated for rabbit eyes. Measurements were made immediately before treatment and at 1, 2, 3, 4, 6, 10, 12, and 24 hr after treatment with forskolin. In monkeys, anesthesia was obtained using ketamine...
10 mg per kg intramuscularly in addition to a drop of proparacaine topically just before IOP measurement. Measurements were made using an applanation pneumotonometer before drug application and at 1, 2, 4, and 8 hr after treatment.

Measurements of IOP in humans were made after topical proparacaine with a Goldmann applanation tonometer immediately before instillation of forskolin and at 1, 2, 3, and 5 hr after treatment.

Topical Forskolin

Forskolin is poorly soluble in water but is readily soluble in ethanol. An initial control study was conducted to determine the effect of topical ethanol alone on rabbit IOP. A 50 μl topical dose in concentrations even as low as 10% ethanol irritated the eyes and caused significant reductions in IOP. To avoid the problems of topical ethanol, forskolin suspensions were made using a balanced salt solution containing 0.5% hydroxypropyl methyl cellulose as a suspending agent. Suspensions were prepared immediately before use by thoroughly mixing finely powdered forskolin with the vehicle in a mortar and pestle. Topical applications of 50 μl were given onto the cornea of the right eye of all animals or subjects, while the left eye received a topical application of a 1% talc suspension in the same vehicle. Talc was used in the control drop in order to approximate the fine particulate nature of the forskolin suspension. The drug was applied well after the effects of topical proparacaine used for baseline IOP measurements had worn off.

Topical suspension concentrations of 0.1%, 1.0%, and 4.0% were each tested in 20 rabbits, and the 1.0% topical suspension was used in 10 monkeys and 10 human subjects; those data have been previously reported. Results of testing with a 0.5% topical suspension in rabbits are reported here.

Tolerance

The potential development of tolerance to the IOP lowering effect of forskolin after short term use was tested for by topical administration of 50 μl of a 1.0% forskolin suspension every 6 hr to the right eye of 10 rabbits, starting at 9 AM daily. The IOP of each rabbit was measured at noon for 15 consecutive days.

Intravitreal Forskolin

A 10 μl volume of forskolin in a 25% ethanol solution was injected into the midvitreous cavity of rabbit eyes using a 25 μl Hamilton syringe and a 30-gauge hubless needle after topical proparacaine. Injections of 0.016 μg, 0.16 μg and 1.6 μg of forskolin were given so that final intraocular concentrations were approximately 10⁻⁸, 10⁻⁷, and 10⁻⁶ M, respectively, assuming a dispersion volume of 4.0 ml. There was no noticeable reflux from the injection site and no conjunctival reaction was noted using this technique. All intravitreal injections containing forskolin were given in the right eye while control injections of 10 μl of 25% ethanol were given in the left eye.

Systemic Forskolin

Forskolin was administered intravenously to five rabbits. Two of these rabbits were anesthetized with ketamine (10 mg/kg) intramuscularly, and three were anesthetized with urethane (5 ml/kg of a 25% solution) intravenously. All rabbits were heparinized with 500 units of heparin sulfate per kg. In the two animals anesthetized with ketamine, blood pressure was monitored with a 22-gauge silastic catheter placed in the left auricular artery. The effect of ketamine was allowed to wear off before forskolin was administered through the left marginal ear vein. Urethanized rabbits had a left femoral artery catheter (22 gauge) placed for blood pressure monitoring and a left femoral vein catheter (22 gauge) placed for drug administration. These animals had the left anterior chamber cannulated with a 23-gauge needle fired by a needle gun. Simultaneous tracings of IOP and blood pressure were made using calibrated transducers and a Sanborn recorder.

Several dilutions of forskolin in absolute alcohol were made; a volume for intravenous injection was prepared by diluting a 10 μl aliquot from an appropriate solution with 0.5 ml of normal saline. This was delivered as an intravenous bolus followed immediately by a 0.5-ml saline flush. Control injections of 10 μl of ethanol in 0.5 ml saline were given randomly. Forskolin doses ranging from 0.005 mg/kg to 0.50 mg/kg were administered.

Tonography

Tonography was performed on six eyes of three rabbits using a 5.5-gm weight and a Mueller electronic tonographic recorder. Animals were anesthetized with ketamine 10 mg/kg intramuscularly 10 min before tonography was performed. Tonographic tracings were made immediately before, and 3 hr after, topical application of a 1.0% forskolin suspension bilaterally. P0 was verified using an applation pneumotonometer. Standard tonographic tables were used to calculate outflow facility.

Aqueous Flow Determination

Measurements of aqueous flow in rabbit eyes treated with a topical 1.0% forskolin were compared to control eyes. The method used was a modification of a flou-
to fluorimetrically. Relative aqueous flow rates were determined in the acetazolamide and forskolin-treated groups, 100% only, were done 3 hr later, and (A, fluorine dextran (MW 40,000) were given bilaterally unilaterally with 50 µl of 1% topical forskolin solution. Two rabbits were treated unilaterally with 50 µl of a 1% topical forskolin suspension.

**Blood Pressure after Topical Forskolin**

Two rabbits were anesthetized with ketamine and five with urethane as described above. A 22-gauge silastic catheter was introduced into the left auricular artery of the ketaminized animals and the left femoral artery of the urethanized animals. Blood pressure was monitored continuously via a transducer and a Sanborn recorder. After stabilization of blood pressure, 50 µl of 1.0% forskolin suspension was placed in the right eye of each animal. IOP was measured every 30 min by applanation pneumotonometry while the blood pressure was monitored continuously for 6 hr. In the urethanized animals, the IOP in the treated eye was monitored continuously by cannulation as described above.

Human brachial artery systolic and diastolic pressure in the right arm were determined in the sitting position using a standard cuff. Pulse rate and blood pressures were recorded at 1, 2, 3, and 5 hr after the topical instillation of 50 µl of a 1.0% forskolin suspension.

**Blood Flow Measurements**

Regional ocular blood flow experiments were performed in 16 rabbits treated unilaterally with 50 µl of a 1.0% topical forskolin suspension. This was accomplished using a modification of the radioactively labeled microsphere technique described by Alm and Bill19 as follows.

Animals were anesthetized with urethane (5 ml/kg of a 25% solution) given intravenously. With the animal in supine position, head straight, and eyes level, 22-gauge silastic catheters were placed in both femoral arteries and one femoral vein. Arterial blood pressure was monitored via a calibrated transducer and a Sanborn recorder. A tracheostomy was performed and the animal ventilated with a Harvard respirator (volume = 22–25 ml, rate = 30–50 per min). The chest was opened by median sternotomy, and the left ventricle was exposed by pericardiotomy. Blood pO2, pCO2, and pH were measured using a blood gas analyzer and the ventilation was regulated so that pH was always maintained between 7.40–7.48. Left intraventricular injections of 0.5 ml of a 1.0% microsphere suspension (specific activity 10 mCi/g) were then performed.

Fifteen-micrometer microspheres of 141 Ce, 57Cr, and 103Ru were used. Four animals received sequential left intraventricular injections of each of the above isotopes just before drug treatment and at various times (0.5–3 hr) after drug treatment. The remaining animals received injections of a single isotope only, from 0.5–8 hr after drug delivery.

Simultaneous with the injection of microspheres, collection of a reference blood sample from the femoral artery was begun at the rate of 2–3 ml per minute and continued for 1 min. Animals then were sacrificed by intracardiac injection of a saturated potassium chloride solution. The eyes were enucleated and dissected immediately. The iris-ciliary body, medial rectus muscle, conjunctiva, choroid, and the anterior 2 mm of the optic nerve were removed and placed in tared vials for weighing and counting. Samples were counted on a Beckman multichannel gamma spectrometer. Three energy windows were used to correspond to the energy peaks of each of the isotopes. Blood flows of all samples were calculated using the reference sample technique, 19...
and ratios of blood flow between treated and contralateral untreated eyes were determined.

Adenylate Cyclase Activity In Vitro

All procedures were conducted on ice except where indicated otherwise. The dissection of ciliary processes from eyes has been described, except that the tissue was dissected and the ciliary tips were collected under Hank’s balanced salt solution (BSS). Rabbit ciliary tissue from 20 eyes was pooled and homogenized in 2.0 ml homogenizing medium (50 mM TRIS HCl, 1 mM DTT, pH 7.5) in a glass-teflon homogenizer using seven strokes of the pestle. The homogenate was centrifuged at 3000 × g at 0–5°C for 5 min and the supernatant was removed. The pellet was resuspended in 2 ml homogenizing medium with seven strokes of the pestle as above. The resulting particulate suspension was mixed immediately with 3.025 ml homogenizing medium, 25 µl were withdrawn for protein determination, and the remaining 5.0 ml divided into 500-µl aliquots that were frozen and stored under liquid nitrogen until use. Final preparation of an aliquot for assay involved thawing, transferring the sample to a 1-ml glass-teflon homogenizer, centrifuging as above, removing the supernatant, and resuspending the pellet in assay medium with seven strokes as above.

Adenylate cyclase was assayed in duplicate at 30°C as described in test tubes containing 100 mM TRIS HCl, pH 7.5, 5 mM MgCl₂, 50 µM creatine kinase, 10 mM creatine phosphate, 2 mM DTT, 0.5 mg/ml bovine serum albumin, 1 mM cyclic AMP, 0.5 mM ATP, 1 µCi α-32P-ATP, 25 µg of particulate protein, and the presence or absence of sodium fluoride, forskolin, or 1-isoproterenol. Recovery of product during the purification procedure was monitored with 3H-cAMP. Human processes were dissected and frozen and stored under BSS in liquid nitrogen until they were used. A crude particulate suspension was prepared as described above, except that 0.5 ml homogenizing medium was used for homogenization and subsequent resuspension, and the resuspension was not diluted further before final resuspension in assay medium. Cultured human ciliary epithelial cells were prepared and assayed as previously described.

Statistics

Values are expressed as the mean ± S.E.M. (n). Statistical analyses were conducted using the Student t test adjusted for paired data, and the Student’s t test for slopes of linear regression. P values of <0.05 were considered significant.

Results

Intravitreal Forskolin

Intravitreal doses of 0.16 µg and 1.6 µg in rabbits produced significant decreases in IOP compared with baseline (Fig. 1). Intravitreal injections of 0.016 µg caused no significant change in IOP, and no significant change in IOP occurred in any of the control eyes.

Topical Forskolin

These data have been reported previously in part. Significant decreases in IOP occurred compared with baseline after topical applications of 0.1%, 0.5%, 1.0% and 4.0% forskolin suspension in rabbits (Fig. 2). Twenty animals were used in each group. The largest effect was seen with the 1.0% dose, with a fall in IOP from 17.4 ± 0.4 mmHg (20) to 13.1 ± 0.4 mmHg...
The duration of significant effect as a function of dose is displayed in Figure 3. No significant IOP change was detected in the contralateral control eyes except in the 1.0% group, which showed a small decrease at 3 hr. A transient superficial hyperemia developed in all treated eyes, lasting for 30–45 min after instillation. No change in pupillary size occurred, and neither flare nor cell was detected by biomicroscopy throughout the experiments.

In monkeys, IOP decreased in the treated eye from 17.7 ± 0.6 mmHg (10) to 14.3 ± 0.8 mmHg (20) at 2 hr (Fig. 4). No significant change in the contralateral control eyes were found. Neither ocular irritation nor aqueous flare nor cell was detected and no significant change in pupillary size occurred during the experiment.

The human data show significant decreases in IOP at 1, 2, 3, and 5 hr after topical administration of 1.0% forskolin suspension (Fig. 4). Mean IOP fell from 14.7 ± 0.8 mmHg (10) to 10.7 ± 1.1 mmHg (10) 2 hr after drug delivery. At the 2-hr interval, and only at this time, a significant decrease was also found in the contralateral control eye. Mean IOP fell in the control eye from 14.5 ± 0.8 mmHg (10) to 12.4 ± 0.8 mmHg (10).

The slope of the linear regression line through the daily mean noon IOP values in the treated eyes was not significantly different from 0 (P = 0.23).

### Tolerance

Topical administration of a 1.0% forskolin concentration to rabbits every 6 hr on 15 consecutive days revealed no significant decrease in the drug's ocular hypotensive effect (Fig. 5). The slope of the linear regression line through the daily mean noon IOP values in the treated eyes was not significantly different from 0 (P = 0.23).

### Tonography

Outflow facility in rabbits measured 3 hr after topical administration of a 1.0% forskolin suspension in six
Table 1. Relative aqueous flow determinations in rabbits

<table>
<thead>
<tr>
<th>Eyes</th>
<th>Aqueous fluorescein concentration (ng/µl)</th>
<th>Significance (t)</th>
<th>Relative decrease in aqueous flow (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.16 ± 0.42</td>
<td>0.012</td>
<td>44%</td>
</tr>
<tr>
<td>Acetazolamide</td>
<td>5.69 ± 0.39</td>
<td>0.011</td>
<td>46%</td>
</tr>
<tr>
<td>Forskolin</td>
<td>5.88 ± 0.44</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Results of t tests comparing aqueous fluorescein in the treated versus control groups.
† Compared with control group.

eyes showed neither suggestion nor statistically significant change in outflow facility compared to the mean baseline value of 0.18 ± 0.04 mmHg (P = 0.41).

Aqueous Flow Determination in Rabbits

The anterior chamber fluorescein–dextran concentrations in the control, acetazolamide-treated, and forskolin-treated eyes are referred to in Table 1. Compared with the nontreated eyes, there was a significant decrease of mean aqueous flow of approximately 46% in the forskolin-treated eyes and approximately 44% in the acetazolamide-treated eyes. The decrease in aqueous flow of 46% in the forskolin treated animals is similar to the decrease in outflow pressure in a group treated similarly and reported earlier using another fluorometric method for flow. These data support the conclusion that the decreased IOP occurring after topical forskolin is caused by a decrease in aqueous inflow without a significant change in outflow facility.*

Effect of Systemic Forskolin on Systolic Blood Pressure and IOP in Rabbits

Intravenous doses as small as 0.0005 mg/kg caused a detectable decrease in systolic blood pressure in rabbits. A 0.5 mg/kg dose given to three rabbits caused a profound and prolonged drop in mean blood pressure, with recovery to baseline after approximately 45 min. A dose–response curve relating drop in mean blood pressure to intravenous forskolin dose was constructed (Fig. 6). With intravenous doses less than 0.5 mg/kg, only small transient volumetric changes in IOP were recorded in the cannulated eyes. Doses of 0.5 mg/kg in three rabbits caused a steady state IOP decrease of 3 to 5 mmHg with the onset at approximately 45 min and recovery to baseline occurring at approximately 200 min.

* A recent study done in the eyes of cynomolgous monkeys after single dose topical 1% suspension of forskolin indicated that aqueous flow measured by noninvasive fluorometry decreased by 35%, while tonographic outflow did not change (personal communication: P. Lee, S. Podos, C. Severin, T. Mittag).

Fig. 6. Linear regression of the maximum decrease in the mean blood pressure as a function of the logarithm of the intravenous forskolin dose (mg/kg) in five rabbits (r = 0.93, P < 0.001).

Effects of Topical Forskolin on Blood Pressure in Rabbits and Humans

Applications of topical 1.0% forskolin suspension caused no change in systolic or mean blood pressure in seven rabbits. Mean baseline blood pressure was 75 ± 4 mmHg (7), and measurements at 0.5, 1.0, 2.0 and 3.0 hr after topical application of drug yielded mean systolic pressures of 77 ± 4 mmHg (7), 78 ± 3 mmHg (7), 8 ± 4 mmHg (7), 76 ± 4 mmHg (7), and 78 ± 3 mmHg (3), respectively. Furthermore, continuously recorded tracings showed no change in blood pressure or pulse rate that could be related to the administration of the drug.

Fig. 7. Mean ratio of iris-ciliary body blood flow in the treated eye versus the untreated contralateral control eye along with the mean ratio of IOP in the treated versus untreated eye in the same rabbits with time. All animals received 50 µl of a 1.0% topical forskolin suspension in the treated eye at time 0, while the other eye received 50 µl of suspension vehicle only.
Fig. 8. Dose response curve for stimulation of rabbit ciliary adenylate cyclase by forskolin. The presence of 1 μM timolol had no effect on cyclase activation by forskolin and yielded a dose response curve identical to the one shown above.

In humans, mean systolic and diastolic blood pressures before treatment with 1.0% topical forskolin were 116 ± 5 and 63 ± 3 mmHg (10), respectively; no significant changes occurred at 1, 2, 3, or 5 hr. Pulse rate was 66 ± 4 min⁻¹ and remained constant during the experiment.

Blood Flow Measurements

The ratio of iris-ciliary body blood flow in the treated versus untreated contralateral eyes is plotted as a function of time after forskolin administration in Figure 7, along with IOP of the same eyes. The mean blood flow in the iris-ciliary body of all untreated eyes was 170 ± 21 (16) gm/min/100 gm fresh tissue. Blood flow in the conjunctiva, rectus muscle, choroid, and optic nerve in the treated eyes was not found to be significantly different from the untreated eyes.

The peak iris-ciliary blood flow occurred at 1 hr, at a time when IOP was not significantly different from baseline, while the maximum IOP decrease occurred at 4 hr, at a time when iris-ciliary blood flow had returned to a value not significantly different from the mean baseline value.

Adenylate Cyclase Activity In Vitro

The dose–response curve for activation of adenylate cyclase from rabbit ciliary processes is shown in Figure 8. The highest forskolin concentrations stimulated adenylate cyclase significantly more than maximal stimulation with either 4 mM NaF or 100 μM 1-isoproterenol. The dose response curve of forskolin activation of cyclase activity in the presence of 10 μM timolol was identical with the curve in the absence of timolol (data not shown). A 25.1-fold activation of rabbit ciliary process adenylate cyclase by forskolin (6.1 μM) occurred in the presence of 4 mM NaF, which was greater than the sum of the responses produced by either activator alone (forskolin, 9.3-fold; NaF, 6.3-fold). Activation of human ciliary process adenylate cyclase is shown in Table 2. The cyclase activation in either freshly dissected human processes or human cultured cells was significantly greater than activation by either 1-isoproterenol or NaF.

Stimulation of rabbit ciliary process adenylate cyclase by isoproterenol in combination with forskolin is synergistic. Activation by 100 μM isoproterenol in the presence of 0.2, 2.0, or 20 μM forskolin was significantly greater than the sum of the responses produced by either activator alone.

Discussion

Forskolin is a diterpene derivative of the plant Coleus forskohlii that acts on the adenylate cyclase catalytic subunit to increase intracellular cAMP, may not require the guanine nucleotide regulatory subunit, and does not require a cell membrane bound protein receptor. Forskolin produces cellular responses dependent on cAMP as a second messenger, and has the ability to potentiate greatly the hormonal activation of adenylate cyclase.10-13 We have reported recently preliminary findings demonstrating dramatic IOP reduction after topical administration in rabbits, monkeys, and humans.16

Intravitreal injection or topical administration of forskolin in suspension significantly reduces IOP in rabbits by reducing aqueous inflow without any increase in outflow facility. Topical administration of forskolin in monkeys and in normal human volunteers also caused significant decreases in IOP.16 During and

---

Table 2. Stimulation of human ciliary adenylate cyclase in vitro

<table>
<thead>
<tr>
<th></th>
<th>Cultured cells*</th>
<th>Ciliary processes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 μM 1-isoproterenol</td>
<td>2.3 ± 0.3 (2)</td>
<td>5.3 ± 0.7 (2)</td>
</tr>
<tr>
<td>4 mM NaF</td>
<td>22.9 ± 2.2 (2)</td>
<td>9.2 ± 2.2 (2)</td>
</tr>
<tr>
<td>61 μM forskolin</td>
<td>57.5 ± 7.0 (2)†</td>
<td>29.7 ± 2.8 (2)†</td>
</tr>
</tbody>
</table>

* Numbers represent ratios of activity in the presence of agent to basal activity.
† Activation by forskolin was significantly greater than isoproterenol or NaF.
after topical ocular delivery of forskolin in rabbits and humans, blood pressure and pulse were measured. No changes occurred. Intravenous administration of forskolin in rabbits produced a steady state decrease in intraocular pressure only after doses large enough to approximate the intraocular concentrations probably achieved after topical administration.

Iris-ciliary body blood flow increased by approximately 2.5-fold 1 hr after topical forskolin, while choroidal flow remained unchanged. This increase probably was caused by a direct vasodilatory effect in the ciliary body. The increase in blood flow and decrease in net aqueous flow mimic the cholera toxin effect.2,3

Forskolin activated adenylate cyclase in rabbit and freshly dissected human ciliary processes, as well as in human cultured ciliary epithelial cells. Timolol did not inhibit this activation, substantiating the finding that forskolin does not work by interaction with the beta-adrenergic receptor. The adenylate cyclase receptor complex consists of a membrane-bound receptor protein, a guanine nucleotide regulatory subunit, and a catalytic subunit. These components of the "second messenger" system are present in the ciliary epithelium in rabbits26–22 and humans.5,23 Increased ciliary adenylate cyclase activity after beta receptor stimulation in both species has been demonstrated,21–22 in accord with beta2 receptor.23 Furthermore, activation of adenylate cyclase induces protein phosphorylation in cultured epithelium, indicating the presence of a cAMP-dependent protein kinase system in this tissue.24 These findings suggest that a complete, functionally coupled, "second messenger" system is present in the ciliary epithelium. In the present work we found that isoproterenol, in combination with forskolin, showed a synergistic effect with cyclase activation in the presence of both drugs being significantly greater than the sum of the activation using either agent alone (Table 3).

A reduction in net aqueous humor inflow mediated by increased cAMP levels in the ciliary epithelium represents a unifying hypothesis concerning the regulation of IOP4 (Fig. 9). Stimulation of the adenylate cyclase complex via beta receptor agonism in the ciliary epithelium can lower IOP.6–9 Topical application of various beta-adrenergic agonists produce changes in aqueous flow (though relatively small), the direction of which seems to vary with the method of measure-

<table>
<thead>
<tr>
<th>Forskolin concentration (µM)</th>
<th>Forskolin alone (pmol/min/mg protein)</th>
<th>Isoproterenol stimulation (pmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>49.7 ± 2.3 (3)</td>
<td>13.7 ± 1.5 (3)</td>
</tr>
<tr>
<td>0.2</td>
<td>124 ± 12 (3)</td>
<td>44.7 ± 6.7 (3)</td>
</tr>
<tr>
<td>2.0</td>
<td>292 ± 18 (3)</td>
<td>93.7 ± 27.4 (3)</td>
</tr>
<tr>
<td>20.0</td>
<td>616 ± 51 (3)</td>
<td>102 ± 23 (3)</td>
</tr>
</tbody>
</table>

* Values represent mean ± SEM (n).
† Isoproterenol stimulation is defined as adenylate cyclase activity in the presence of 100 µM isoproterenol minus the activity in the absence of isoproterenol for each forskolin concentration. This represents the component of only isoproterenol stimulation in the presence of forskolin.
‡ Isoproterenol stimulation in the presence of forskolin is greater than the stimulation in the absence of forskolin (P < 0.05).

Fig. 9. The proposed relationship between exogenous and endogenous factors on the regulation of intraocular pressure via modulation of aqueous inflow by cyclic AMP.

Table 3. Effect of forskolin on isoproterenol stimulation of rabbit adenylate cyclase
Fluorometric determinations of aqueous flow have been done after topical fluorescein, iontophoresis, oral or intravenous fluorescein, posterior chamber fluorescein, or after intravitreal injection of fluorescein labelled dextran. The latter technique allows determinations over a longer period of time, can disentangle multiple phases (eg, an initial increase followed by a decrease), and can demonstrate relatively small changes in flow induced by catecholamines that otherwise may go undetected. Maurice has demonstrated, for example, that topical epinephrine causes first a slight transient increase in flow, followed by a prolonged decrease.

Work in our laboratory demonstrated that cholera toxin, a potent irreversible stimulator of adenylate cyclase, stimulates adenylate cyclase in rabbit and human ciliary epithelium and lowers IOP by reducing aqueous flow in rabbits when administered in minute (10^-10 M) doses by either intravitreal injection or close arterial infusion. The gonadotropins—hCG and FSH—which are glycoprotein stimulators of cAMP production also decrease IOP and aqueous flow in either normal or oophorectomized rabbits by either intravitreal or by systemic intramuscular injection. Thus, beta-adrenergic agonists, certain glycoprotein hormones, cholera toxin, fluorescein, and forskolin all stimulate adenylate cyclase, albeit by different molecular pathways. Catecholamines and cholera toxin have been shown previously to reduce IOP in steady state by lowering aqueous inflow. Certain clinical syndromes such as myotonic dystrophy, choriocarcinoma, and pregnancy are associated with high serum gonadotropin levels and low IOP. We now have confirmed a dramatic reduction in aqueous flow and IOP with forskolin. Exogenously applied substances and endogenous circulating hormones may regulate aqueous inflow (and thus IOP) via a final common pathway, the adenylate cyclase receptor complex in the ciliary epithelium.

The mechanism by which elevated intracellular cAMP levels lead to decreased aqueous production is not known. One hypothesis is introduced here. The effect of cholera toxin in the gut, cochlea, and choroid plexus has been investigated. In each case, stimulation of adenylate cyclase in epithelial cells produces bulk flow of water and electrolytes from the basal side of the cell out through the cellular apex in accord with the polarity of the cells. In the eye, however, the apices of the pigmented and nonpigmented ciliary epithelia are pointed toward each other as a result of the invagination of the optic vesicle during embryologic development, as reviewed by Hogan et al. The nonpigmented epithelium thus has its base toward the aqueous compartment and its apex toward the blood. In addition, histochemical localization of adenylate cyclase in the ciliary body apparently is restricted largely to the nonpigmented epithelium with little or no activity in the pigmented epithelium. Stimulation of the enzyme in the nonpigmented layer may promote reabsorption of fluid from the posterior chamber and secretion into the ciliary stroma, thereby decreasing "net" aqueous inflow. Vascular effects on secretion secondary to increased ciliary body blood flow cannot yet be excluded.

Forskolin and its analogues may represent a new class of antiglaucoma drugs differing in its molecular mechanism from any previously used drug. Its effect on IOP should be additive with other glaucoma drugs, owing to its unique mode of action, and may even potentiate the effect of certain drugs, eg, epinephrine, which function (partially) through beta-receptor agonism. Tachyphylaxis or tolerance may not occur because forskolin's action does not involve the cell surface receptor. Slight modification of the molecule may increase its water solubility and corneal penetration, reducing the dose delivered to the surface of the eye and enhancing its effectiveness. More work in this area is warranted to determine forskolin's potential therapeutic usefulness.

**Key words:** forskolin, adenylate cyclase, glaucoma, aqueous humor formation, intraocular pressure, cyclic AMP, blood flow, ciliary epithelium

**References**


