# Effect of Topically Applied Forskolin on Aqueous Humor Dynamics in Cynomolgus Monkey

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**Topical administration of a 1% forskolin suspension significantly reduced intraocular pressure in cynomolgus monkey eyes. The fall in intraocular pressure was associated with a significant ($P < 0.01$) decrease in aqueous humor flow measured by a fluorophotometric technique. No significant change was found in tonographic outflow facility or pupillary diameter. A loss of effect on intraocular pressure to subsequent doses of 1% forskolin suspension occurred in cynomolgus monkeys by the third day of twice-a-day treatment. Invest Ophthalmol Vis Sci 25:1206–1209, 1984**

Forskolin is a diterpene derivative of the plant *Coleus forskohlii*. Ethanolic extracts from the roots of the plant have marked hypotensive and antispasmolytic activity. The major diterpene isolated from the Coleus roots is a 7-acetoxy-1$\alpha$,6$\beta$9$\alpha$-trihydroxy derivative of manoyl oxide, which itself is the 8,13$\alpha$-epoxy-14-en-11-one derivative of the labdane family of diterpenes, named forskolin. Forskolin is a unique activator of adenylate cyclase.[1]

Caprioli and Sears[2] reported that topical ocular application of forskolin lowers intraocular pressure in rabbit, monkey, and humans and reduces aqueous flow in rabbit. They suggested that forskolin and its analogues may be a new class of drug active against glaucoma. Bartels, Lee, and Neufeld[3] noted that in rabbits, intracameral injection of forskolin lowers intraocular pressure and increases outflow facility as measured by a constant pressure perfusion technique.

We studied the effects of a single application of forskolin on intraocular pressure, outflow facility, aqueous humor flow, aqueous humor flare, and pupillary diameter in order to clarify its mode of action in monkeys. In addition, we investigated the effect of multiple doses of forskolin on intraocular pressure.

## Materials and Methods

Adult cynomolgus monkeys, 3–5 kg, were studied. The monkeys were kept in a primate chair throughout each experiment. A drop of a local anesthetic (0.5% proparacaine hydrochloride) was applied to the eye before intraocular pressure and outflow facility measurements. The intraocular pressure, outflow facility, and aqueous humor flow were measured in animals lightly anesthetized with ketamine hydrochloride, 5–10 mg/kg given intramuscularly, about 5 min before each measurement. Pupillary diameters were measured with a millimeter ruler in normal room light. The aqueous humor flare in the anterior chamber was assessed by slit-lamp examination and rated from 0 to 3 (aqueous flare: 0 = no Tyndall effect, 1+ = slight Tyndall effect, 2+ = moderate to dense Tyndall effect, 3+ = dense Tyndall effect with fibrin clots; cellular response: 0 = no cells apparent, 1+ = few cells, 2+ = many cells, 3+ = cell clumps).

Forskolin (Calbiochem Behring Corporation; La Jolla, CA) was suspended in isotonic buffered saline solution containing 0.5% methylcellulose, pH 7.2. Thorough mixing of the suspension was carried out initially and before each use using a high speed vortex mixer. For all experiments, a 50 $\mu$l drop of 1% forskolin was applied to one eye, either right or left at random. An equal volume of diluent was administered to the fellow control eye.

Intraocular pressure was measured with a calibrated Alcon pneumatonometer (Fort Worth, TX). Baseline intraocular pressures were measured twice prior to 1% forskolin administration. Repeat intraocular pressure measurements were made at 0.5, 1, 2, 3, 4, 5, and 6 hr after the drug administration.

Tonography was performed with an Alcon EDT-103 tonography unit. Baseline outflow facility was determined between 9 AM and 10 AM, 2 hr before administration of 1% forskolin. Tonography was repeated 1 hr after drug administration. Facility of outflow values were approximated from the 1955 Friedenwald tables.

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Supported in part by grants EY-03651, EY-02619, and EY-01867 from the National Eye Institute, Bethesda, Maryland, and by an unrestricted grant from Research to Prevent Blindness, Inc.

Submitted for publication: January 10, 1984.

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**Table 1.** The effect of 1% forskolin on intraocular pressure in 14 cynomolgus monkeys

| | *Mean intraocular pressure + SE (mmHg)* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *0 hr* | *0.5 hr* | *1 hr* | *2 hr* | *3 hr* | *4 hr* | *5 hr* | *6 hr* |
| Forskolin | 17.1 ± 0.4 | 15.7 ± 0.4* | 14.7 ± 0.4* | 15.8 ± 0.5† | 15.1 ± 0.6* | 15.6 ± 0.6* | 16.2 ± 0.6* | 16.4 ± 0.7‡ |
| Diluent | 16.9 ± 0.3 | 17.5 ± 0.4 | 17.0 ± 0.5 | 17.1 ± 0.5 | 17.1 ± 0.5 | 17.4 ± 0.4 | 17.6 ± 0.5 | 17.1 ± 0.7 |

* Significant difference between eyes treated with forskolin and fellow control eyes, paired t-test, $P < 0.001$.

† Significant difference between eyes treated with forskolin and fellow control eyes, paired t-test, $P < 0.005$.

‡ Significant difference between eyes treated with forskolin and fellow control eyes, paired t-test, $P < 0.01$.

Aqueous humor flow was estimated using a fluorophotometric technique.[4-6] A model 360 Haag Streit slit lamp (Berne, Switzerland) was modified as described by Waltman and Kaufman[7] to convert it to an objective fluorophotometer. The lamp and microscope arm were locked at an angle of 60 deg for measurements. The fluorescein iontophoresis was done at 4 PM and fluorescence measurements were made from 9 AM to 1 PM on the following day. The iontophoresis was carried out in the central 4 mm of the cornea with an electrode of 10% fluorescein in 2% agar. A current of 200 μA was used for 5–7 min. Fluorophotometric measurements of the cornea and anterior chamber were repeated at about 45-min intervals. Four or five such measurements were made. Following these baseline measurements, on another day, 1% forskolin was applied topically to one eye of each monkey at about 9 AM. The iontophoresis was carried out at 4 PM on the preceding day as described above. Fluorophotometric measurements were taken from 0.5 to 4 hr after instillation of 1% forskolin. For each animal, the natural logarithm of F (Ine F) was plotted versus time. The lines of best fit and their slopes were calculated by the least-squares method. The value of A used for each eye was midway between the absolute values of the slopes of the best-fit line for anterior chamber and best-fit line for cornea. The term A is the absolute magnitude of the slope of the line for Ine F versus time for the cornea or for the anterior chamber. The value of Fc/Fa (Fc and Fa are fluorophotometric readings of cornea and anterior chamber, respectively) at 1 hr after 1% forskolin administration was determined from the graph of each animal. Values of 106 μl[8] for anterior chamber volume and 50 μl for cornea volume in monkeys (unpublished data, M. E. Yablonski and J. B. Serle) were used in the calculation.

The intraocular pressure lowering effect of forskolin was tested after multiple short term use. A 50-μl drop of 1% forskolin suspension was applied topically to one eye of each monkey at 9 AM and 5 PM daily for five days. At the same time, an equal volume of diluent was administered to the fellow control eye. Intraocular pressures were measured at 1, 2, 4, 6, and 8 hr after the morning administration of forskolin on the first, third, and fifth day of treatment. The 1% forskolin suspension was prepared fresh on days 1 and 5. These experiments adhered to the ARVO Resolution on the Use of Animals in Research.

## Results

Topical administration of a 50-μl drop of 1% forskolin suspension to monkey eyes resulted in reduction of intraocular pressure (Table 1). The mean intraocular pressure was reduced significantly ($P < 0.01$) for at least 6 hr after forskolin administration in 14 cynomolgus monkeys. The greatest hypotensive response was observed at 1 hr after the application of forskolin.

Tonometry 1 hr after the unilateral administration of 1% forskolin confirmed the reduction of intraocular pressure in eight monkeys. Intraocular pressure in the treated eye was significantly ($P < 0.001$) lower than in the fellow control eye. Tonographic outflow facility was similar in forskolin-treated eyes and control diluent-treated eyes before and after therapy (Table 2).

After unilateral administration of 1% forskolin, the aqueous humor flow (Table 2) in the eight treated

**Table 2.** The effect of 1% forskolin on aqueous humor dynamics in 8 cynomolgus monkeys

| | *Intraocular pressure (mmHg)* | *Outflow facility (μl/min/mmHg)* | *Aqueous flow (μl/min)* |
|---|---|---|---|
| Forskolin | | | |
| Baseline | 16.9 ± 0.4 | 0.54 ± 0.07 | 1.76 ± 0.07 |
| Treated | 14.1 ± 0.7* | 0.57 ± 0.04 | 1.28 ± 0.11† |
| Diluent | | | |
| Baseline | 16.8 ± 0.4 | 0.56 ± 0.08 | 1.82 ± 0.24 |
| Treated | 17.5 ± 0.5 | 0.57 ± 0.04 | 1.98 ± 0.19 |

Values shown are mean ± SE.

* Significantly different as compared with baseline ($P < 0.01$) and to fellow control eyes ($P < 0.001$) paired t-test.

† Significantly different as compared with baseline ($P < 0.01$) and to fellow control eyes ($P < 0.01$), paired t-test.

**Table 3.** Intraocular pressure in four monkeys during a 5-day period of topical administration of a 1% forskolin suspension twice daily at 9 AM and 5 PM

| | | *Mean intraocular pressure + SE (mmHg)* | | | | |
|---|---|---|---|---|---|---|
| | | *10 AM* | *11 AM* | *1 PM* | *3 PM* | *5 PM* |
| Baseline | Treated eye | 17.9 ± 0.4 | 17.9 ± 0.4 | 17.5 ± 0.3 | 17.6 ± 0.4 | 18.5 ± 0.4 |
| | Control eye | 18.1 ± 0.4 | 17.9 ± 0.4 | 17.4 ± 0.3 | 17.6 ± 0.3 | 18.4 ± 0.4 |
| Day 1 | Treated eye | 13.8 ± 1.5* | 15.5 ± 1.0† | 16.8 ± 0.7* | 16.8 ± 0.5* | 16.8 ± 0.5† |
| | Control eye | 18.0 ± 1.3 | 19.0 ± 0.7 | 19.0 ± 0.4 | 18.8 ± 0.5 | 19.0 ± 0.7 |
| Day 3 | Treated eye | 16.3 ± 1.1 | 16.5 ± 0.5 | 17.5 ± 0.7 | 16.3 ± 0.8 | 16.8 ± 0.3 |
| | Control eye | 17.3 ± 1.1 | 17.3 ± 0.5 | 17.3 ± 0.5 | 16.8 ± 1.3 | 17.0 ± 0 |
| Day 5 | Treated eye | 17.5 ± 0.5 | 17.8 ± 0.3 | 18.7 ± 0.8 | 15.5 ± 1.4 | 16.3 ± 1.2 |
| | Control eye | 17.8 ± 0.5 | 18.5 ± 0.7 | 18.5 ± 0.9 | 16.8 ± 1.1 | 17.8 ± 1.3 |

* Significant difference between eyes treated with forskolin and fellow control eyes, paired t-test, $P < 0.005$.

† Significant differences between eyes treated with forskolin and fellow control eyes, paired t-test, $P < 0.02$.

eyes was significantly ($P < 0.01$) lower than in the fellow control eyes and baseline values. Baseline aqueous humor flow was similar in the treated eyes and fellow control eyes. The mean aqueous humor flow in the treated eyes was reduced 35% as compared with fellow control eyes and 27% as compared with baseline of the same eye.

Pupillary diameter was not altered nor was aqueous humor flare seen in any of the monkey eyes at any time after topical application of 1% forskolin.

In the multiple dose experiment, baseline tonometry, taken at 10 AM, 11 AM, 1 PM, 3 PM, and 5 PM daily for up to 2 days prior to treatment, indicated no significant difference between the intraocular pressures of the treated and fellow control eyes of four monkeys. The intraocular pressure of the treated eyes was significantly lower ($P < 0.02$) than the fellow control eyes after the initial topical application of 1% forskolin suspension on the first day of treatment. The intraocular pressure of the treated eyes was not significantly different from the fellow control eyes on the third day and fifth day of twice-a-day treatment (Table 3).

## Discussion

The results obtained in the present study show that a 50-$\mu$l drop of 1% forskolin suspension applied to monkey eyes produced a significant unilateral reduction of intraocular pressure. Comparing the forskolin-treated eyes with the diluent-treated eyes, a 35% reduction in aqueous humor flow was measured by fluorophotometry and a 27% reduction comparing the forskolin-treated eyes with their baseline values. In Caprioli and Sears' study,[2] it is noted that the fall in intraocular pressure after 1% forskolin administration to rabbit eyes was associated with a 40% reduction in aqueous humor flow and was not associated with a detectable change in outflow facility. Thus, based on the results of Sears and on the present finding, the effect of forskolin may be presumed to be largely due to an effect on aqueous humor production although changes in outflow facility also could be a component of the forskolin effect.

Topical ocular administration of a 1% forskolin suspension to rabbits every 6 hr on 15 consecutive days revealed no significant decrease in the drug's ocular hypotensive effect.[9] However, the results of the present preliminary experiment in four cynomolgus monkeys demonstrated that there was no longer any statistical difference in intraocular pressure between treated eyes and fellow control eyes by the third through fifth day of twice-a-day, topical administration of a 1% forskolin suspension. The suggested development of tolerance in monkeys but not in rabbits[9] may reflect a species difference. If similar tolerance develops with respect to the ocular hypotensive effects of forskolin in humans, it would preclude its use in the treatment of chronic glaucoma. The adenylate cyclase receptor complex consists of a membrane-bound receptor protein, a guanine nucleotide regulatory subunit, and a catalytic subunit. The components of this system can be found in the ciliary epithelium. Beta-receptor adrenergic agonists stimulate ciliary adenylate cyclase activity.[10] Furthermore, activation of adenylate cyclase induces protein phosphorylation in cultured human ciliary epithelium indicating the presence of a protein kinase system in this tissue.[11] Forskolin activates adenylate cyclase while not requiring the cell surface receptor nor the guanine nucleotide regulatory subunit of the enzyme and probably acts via the catalytic subunit of adenylate cyclase.[1] The stimulation of adenylate cyclase by isoproterenol in vitro is potentiated in the presence of forskolin.[9] Recently, Downs and Aurbach[12] sug-

gested that the guanine nucleotide regulatory protein (G protein) enhances forskolin activation of adenylate cyclase. Insel et al[13] reported that forskolin is a potent and highly efficacious activator of platelet adenylate cyclase. Forskolin not only produces additive or more than additive enhancement with activators of platelet adenylate cyclase activity, but it also increases specific epinephrine-induced inhibition of adenylate cyclase activity in platelet membranes. Forskolin may activate adenylate cyclase independent of a cell surface receptor and of the G protein, but the exact locus of forskolin action and the role of the G protein and catalytic unit has not been elucidated.

It is difficult to reconcile the aqueous humor dynamics effect of topical forskolin with its action on adenylate cyclase. Intracameral cyclic AMP has been found to increase outflow facility in rabbits,[14] whereas beta adrenergic antagonists, which block norepinephrine-induced accumulation of cyclic AMP in the ciliary body, reduce aqueous production. Intracameral forskolin increases outflow facility in rabbit eyes,[3] whereas it reduces aqueous flow by topical application in monkeys. The latter finding is in accord with effects of cholera toxin, which activates cyclic AMP sensitive systems and causes a dramatic decrease in intraocular pressure when delivered to the ciliary processes from either side of the blood–aqueous barrier (from the blood supply or from the vitreous) and which is associated with a drastically reduced aqueous flow.[15]

Rushton[16] points out that Caprioli and Sears attribute the fall in intraocular pressure following forskolin to reduced secretion of aqueous humor associated with increased cyclic AMP levels in the ciliary-process epithelial cells. Their rationalization that secretion by the cells increases but is directed into the ciliary-process stroma and not into the chamber of the eye, because the cells were inverted during the formation of the eye, is supported by the morphologic appearance of the nonpigmented epithelium, but is not supported by the mucosal (apical) secretion of other epithelia, such as the choroid plexus, in which the direction of cyclic AMP and $Na^+$, $K^+$-ATPase stimulated secretions is the same.[17] Furthermore, previous detailed analyses of the mechanism whereby aqueous humor formation and cyclic AMP formation are stimulated by $\beta$-adrenergic agonists,[18] although the effects are small, and decreased by $\beta$-adrenergic blockers are not compatible with their hypothesis. Further studies are needed to resolve these apparent inconsistencies.

**Key words:** forskolin, monkey, intraocular pressure, outflow facility, aqueous humor flow, adenylate cyclase

## References

1. Seamon KB and Daly JW: Forskolin: a unique diterpene activator of cyclic AMP-generating systems. J Cyclic Nucl Res 7:201, 1981.
2. Caprioli J and Sears M: Forskolin lowers intraocular pressure in rabbits, monkeys, and man. Lancet 1:958, 1983.
3. Bartels SP, Lee SR, and Neufeld AH: Forskolin stimulates cyclic AMP synthesis, lowers intraocular pressure and increases outflow facility in rabbits. Curr Eye Res 2:673, 1982/1983.
4. Jones RF and Maurice DM: New methods of measuring the rate of aqueous flow in man with fluorescein. Exp Eye Res 5:208, 1966.
5. Yablonski ME, Zimmerman TJ, Waltman SR, and Becker B: A fluorophotometric study of the effect of topical timolol on aqueous humor dynamics. Exp Eye Res 27:135, 1978.
6. Schenker HI, Yablonski ME, Podos SM, and Linder L: A fluorophotometric study of epinephrine and timolol in human subjects. Arch Ophthalmol 99:1212, 1981.
7. Waltman SR and Kaufman HE: A new objective slit lamp fluorophotometer. Invest Ophthalmol 9:247, 1970.
8. Johnson SB, Passmore JA, and Brubaker RF: The fluorescein distribution volume of the anterior chamber. Invest Ophthalmol Vis Sci 16:633, 1977.
9. Caprioli J, Sears M, Bausher L, Gregory D, and Mead A: Forskolin lowers intraocular pressure by reducing aqueous inflow. Invest Ophthalmol Vis Sci 25:268, 1984.
10. Neufeld AH and Sears ML: Cyclic-AMP in ocular tissues of the rabbit, monkey, and human. Invest Ophthalmol 13:475, 1974.
11. Coca-Prados M, Kondo K, and Sears ML: Protein phosphorylation in cultured human ciliary epithelia in response to activators of adenylate cyclase, cyclic AMP and analogues. *In* Glaucoma Update II, International Glaucoma Symposium, Carmel, California, October 22–27, 1982, Krieglstein GK and Leydhecker HW, editors. Heidelberg, Springer-Verlag, 1983, pp. 1–6.
12. Downs RW Jr and Aurbach GD: The effects of forskolin on adenylate cyclase in S49 wild type and cyc$^-$ cells. J Cyclic Nucl Res 8:235, 1982.
13. Insel PA, Stengel D, Ferry N, and Hanoune J: Regulation of adenylate cyclase of human platelet membranes by forskolin. J Biol Chem 257:7485, 1982.
14. Neufeld AH, Dueker DK, Vegge T, and Sears ML: Adenosine 3′,5′-monophosphate increases the outflow of aqueous humor from the rabbit eye. Invest Ophthalmol 14:40, 1975.
15. Gregory D, Sears M, Bausher L, Mishima H, and Mead A: Intraocular pressure and aqueous flow are decreased by cholera toxin. Invest Ophthalmol Vis Sci 20:371, 1981.
16. Rushton A: Cyclic AMP and intraocular pressure. Lancet 2:737, 1983.
17. Feldman AM, Epstein MH, and Brusilow SW: Role of cyclic AMP in cerebrospinal fluid production. *In* Neurobiology of Cerebrospinal Fluid, Wood JH, editor. New York, Plenum Press, 1980, pp. 17–25.
18. Brubaker RJ: The flow of aqueous humor in the human eye. Trans Am Ophthalmol Soc 80:391, 1983.