The effect of timolol maleate on the disruption of the blood-aqueous barrier in the rabbit eye

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A disruption of the blood-aqueous barrier in rabbit eyes was elicited by use of topical prostaglandin E₂ (PGE₂), infrared irradiation of the iris, or by subcutaneous α-melanocyte-stimulating hormone (α-MSH). The aqueous flare provoked was measured quantitatively with a photoelectric instrument. The effect of the (topical) β-adrenergic antagonist timolol maleate on the breakdown of the blood-aqueous barrier was tested. Timolol applied topically in very large doses had no effect on exogenously administered PGE₂. However, even in a very small concentration applied topically, timolol reduced the flare response to both infrared irradiation and α-MSH. These results support the theory that the effect of α-MSH and infrared irradiation on the blood-aqueous barrier is dependent on intact β-adrenergic receptor sites.

Key words: blood-aqueous barrier, prostaglandin, infrared irradiation, α-MSH, rabbit eye, timolol

Infrared irradiation of the iris and α-MSH given subcutaneously cause a breakdown of the blood-aqueous barrier in rabbit eyes. Like many other traumatic stimuli to the eye, infrared irradiation is known to exert its effect via enhanced prostaglandin (PG) synthesis and release in the eye,1, 2 since the action of irradiation can be inhibited by aspirin3 and indomethacin,3, 4 which specifically block prostaglandin synthetase.5 However, the barrier-disturbing action of α-MSH cannot be prevented by the PG inhibitor indomethacin, and thus α-MSH is supposed to exert its effect in a way different from that of traumatic stimuli like infrared irradiation.4 It has further been suggested that the α-MSH action on the blood-aqueous barrier is due merely to enhanced β-adrenergic activity, since the β-adrenergic antagonist propranolol given intravenously totally abolishes the flare response to α-MSH.6 The effect of infrared irradiation of the iris seems, however, to be conditioned by α- as well as β-adrenergic activity, since both propranolol and the α-adrenergic antagonist phentolamine, given intravenously, are capable of reducing the flare response to infrared irradiation.6

Exogenously applied PGE₂ mimics the effect of traumatic stimuli on the blood-aqueous barrier, an effect that is unaffected by indomethacin4 as well as by adrenergic blockers,6 indicating that PG synthesis and action are linked in the chain of reactions after adrenergic receptor stimulation.

The purpose of the present study was to investigate whether the topically applied β-adrenergic antagonist timolol maleate is capable of inhibiting the disruption of the blood-aqueous barrier in the rabbit eye elicited by the three stimuli mentioned above. Timolol maleate is a potent β-adrenergic blocking drug, several times more...
active than propranolol. Timolol inhibits both $\beta_1$ and $\beta_2$ receptors and has no intrinsic sympathomimetic or local anesthetic activity. The drug has been shown to lower intraocular pressure in normal volunteers and in patients with glaucoma.

Materials and methods

Animals. Adult pigmented rabbits (1.5 to 3.5 kg) of mixed strains were used. They were given pellets and water ad libitum.

Chemical preparations. Prostaglandin $E_2$ (PGE$_2$) (The Upjohn Co., Kalamazoo, Mich.) was dissolved in 10 mg/ml ethanol, and saline was added to give a solution containing 0.5 mg/ml. $\alpha$-MSH (Ciba Pharmaceutical Co.) was freshly dissolved in saline (100 $\mu$g/ml). Timolol maleate (Blocadren; Merck, Sharp & Dohme) was used in concentrations of 5, 2.5, 0.025, 0.005, and 0.0025 mg/ml.

Aqueous flare response. The course of the barrier damage was followed by measuring photoelectrically the aqueous flare response seen in the anterior chamber, which reflects the protein leakage across the blood-aqueous barrier. The flare was measured in arbitrary units.

Pupillary diameter. In the series in which infrared irradiation was tested, the pupillary diameter was recorded with a clear plastic ruler.

Blood pressure. The blood pressure was registered with a modification of the apparatus described by Kylin. An artery of the rabbit’s ear was chosen for the measurements.

Corneal sensation. The corneal sensation was tested with wetted cottonwool.

Experiments

Disruption of blood-aqueous barrier. This was elicited with the use of the following three irritants: (1) infrared irradiation of the iris for 2 min, (2) $\alpha$-MSH (20 $\mu$g/kg body weight) given subcutaneously, and (3) 2.5 $\mu$g of PGE$_2$ locally applied to the cornea.

The testing of the effect of the $\beta$-adrenergic antagonist timolol on the breakdown of the blood-aqueous barrier caused by the three irritants was performed according to the following scheme. No anesthesia was needed for any of the experiments.

Infrared irradiation. One group of rabbits (nine animals) was first tested to determine whether a dose of 50 $\mu$l of 0.005% timolol given topically onto the right eye every 15 min for 1½ hr previous to infrared irradiation of the iris of both eyes affected the protein leakage in any of the eyes.

The dose of timolol tested was then gradually reduced to determine the minimum dose sufficient to affect the aqueous flare response to infrared irradiation. The doses of timolol (given once onto the right eye 1½ hr previous to irradiation of both eyes) tested were 50 $\mu$l of 0.25% timolol (eight animals), 50 $\mu$l of 0.0025% timolol (two ani-
Infrared irradiation (IR) and 0.25% timolol (T)

![Graph showing aqueous flare response](image_url)

**Fig. 2.** Aqueous flare response (AFR) to infrared irradiation of the iris of both eyes after prior topical treatment of the right eye with 50 µl of 0.25% timolol (eight rabbits). Aqueous flare response to infrared irradiation of the left eye when no pretreatment had been given was tested on another occasion for control. Ordinate: flare values ± S.E. (arbitrary units).

...mals), 50 µl of 0.0005% timolol (eight animals), and 50 µl of 0.00025% timolol (two animals). For control, the effect of 2 min infrared irradiation of the iris of the left eye in each group of animals had been determined on an earlier occasion.

α-MSH. One group of rabbits (eight animals) was tested to determine whether 50 µl of 0.5% timolol given topically onto the right eye every 30 min during the hour previous to subcutaneous α-MSH affected the protein leakage in any eye. In another group (six animals) 50 µl of 0.0005% timolol were administered once to the right eye 1 hr before administration of α-MSH.

For control, the aqueous flare response in each group to the same dose of α-MSH given subcutaneously had been measured 3 weeks earlier.

**Results**

**Aqueous flare response and pupil size.** Time course of the aqueous flare response to all the three stimuli was unaffected by timolol.

**Infrared irradiation.** The response to infrared irradiation was significantly (H₀: p < 0.01) reduced by topically applied timolol in a concentration of 0.0005% to 0.5%. The flare was reduced to the same extent even in the untreated contralateral eye (Figs. 1 to 3).

Timolol in a concentration of 0.00025% had no inhibiting effect on the aqueous flare response (two animals).

The pupillary miosis after infrared irradiation was slightly reduced by 0.0005% to 0.5% timolol, and the size of the pupil went back to normal somewhat earlier.

**α-MSH.** Timolol significantly inhibited the aqueous flare response to α-MSH when given in a dose of both 0.0005% (Fig. 4)
Fig. 3. Aqueous flare response (AFR) to infrared irradiation of the iris of both eyes after prior topical treatment of the right eye with 6 × 50 μl of 0.5% timolol (nine rabbits). □, Aqueous flare response to infrared irradiation of the left eye when no pretreatment had been given was tested on another occasion for control. Ordinate: flare values ± S.E. (arbitrary units).

Fig. 4. Aqueous flare response (AFR) to subcutaneous α-MSH after prior topical treatment of the right eye with 50 μl of 0.0005% timolol (six animals). •, ▲, ▼, Aqueous flare responses to α-MSH when no pretreatment had been given was tested on another occasion for control. Ordinate: flare values ± S.E. (arbitrary units).

\( H_0: p < 0.05 \) and 0.5% (Fig. 5) \( H_0: p < 0.01 \). Timolol had a significant effect in the untreated contralateral eye as well.

\( PGE_2 \). Topically applied timolol had no significant effect (Wilcoxon’s test for paired differences. \( H_0: p > 0.10 \)) on the aqueous flare response to 2.5 μg of \( PGE_2 \) (Fig. 6).

**Blood pressure and corneal sensation.** The blood pressure and corneal sensation was unaffected by 0.5% timolol in all four rabbits.
Fig. 5. Aqueous flare response (AFR) to subcutaneous α-MSH after prior topical treatment of the right eye with 2 × 50 μl of 0.5% timolol (eight rabbits). •, ▲. Aqueous flare responses to α-MSH when no pretreatment had been given was tested on another occasion for control. Ordinate: flare values ± S.E. (arbitrary units).

Prostaglandin E₂ (PGE₂) and 0.5% timolol (T)

Fig. 6. Aqueous flare response (AFR) to 2.5 μg of PGE₂ given topically onto the right eye after prior topical treatment of the right eye with 6 × 50 μl of 0.5% timolol (eight rabbits). o, ▲. Aqueous flare response to PGE₂ to the left eye when no pretreatment had been given was tested on another occasion for control. Ordinate: flare values ± S.E. (arbitrary units).

Discussion

There had been evidence that the aqueous flare response to different stimuli to the eye was mediated via α- and β-receptors. The existence of both α- and β-adrenergic receptors in the rabbit iris–ciliary body was shown by Neufeld and Page and by Green and Griffin. In an attempt to localize β-adrenergic receptors in the anterior segment of the eye, Dafna et al. injected a...
fluorescent analogue of propranolol into the carotid arteries of albino rabbits and found that the fluorescence was noted mainly in the ciliary epithelium. It has been claimed that timolol has extremely high potency as a β-adrenergic receptor antagonist because of its great ability to displace the radioligand from the β-adrenergic receptor. Furthermore, Vareilles et al. found that timolol in a concentration as small as 0.0001% considerably reduced the effect of isoproterenol in the rabbit eye and that this effect of timolol was most likely related to its β-adrenergic blocking activity. The extremely high potency of timolol as a β-adrenergic receptor antagonist has also been described by Scriabine et al., who showed that as little as 9 ng/ml timolol were enough to block the positive isotropic effect of isoproterenol on isolated cat papillary muscle. This is in line with the results of our study, which show that timolol applied topically in a dose as small as 0.0005% reduces the aqueous flare response in the rabbit eye caused by infrared irradiation or subcutaneous α-MSH. This small dose of timolol was even capable of reducing the flare response to the same extent in the untreated contralateral eye.

Timolol slightly reduced the miosis evoked by infrared irradiation, an effect that was seen even in the contralateral untreated eyes. Contralateral effects of timolol have also been described by Radius et al., who found a significant decrease of the intraocular pressure in the contralateral untreated eyes of rabbits. Contralateral effects have also been observed in humans treated with timolol. These contralateral effects may be due to some central locus of drug action, compatible with the fact that the flare reduction of the timolol-treated eye was not significantly different from that of the contralateral untreated eye in any of the α-MSH or irradiation series. There have also been reports of effects on the central nervous system in patients treated with timolol. Contralateral response may of course also be due to the action of systemically absorbed drug. This theory, however, is not supported by the dosage-response studies on timolol action on the flare response to infrared irradiation in the present study.

The effect of timolol on the flare response to infrared irradiation and to α-MSH cannot be due merely to a blood pressure-reducing action of timolol, since not even the highest dose of timolol tested reduced the blood pressure in any rabbit. Furthermore, the highest dose of timolol tested was not able to change the PG-induced barrier damage.

The barrier-damaging action of infrared irradiation is known to be inhibited by pretreating the corneas with local anesthesia. Many β-blocking agents have local anesthetic properties, a fact that must be kept in mind when their action on infrared irradiation is tested. Timolol has been said to have no local anesthetic action, but recently corneal anesthesia after timolol therapy in patients has been reported. However, this has only been shown in patients who have been using the drug for a minimum of 3 months, and in our study the corneal sensation was not disturbed even by the highest timolol dose. The action of α-MSH has never been shown to be reduced by pretreatment with local anesthesia.

The results of this study confirm the theory that β-adrenergic receptors play some part in the aqueous flare response elicited by infrared irradiation and α-MSH and that β-adrenergic receptor stimulation is linked in the chain of reactions before PG synthesis and release.

REFERENCES
6. Bengtsson E: Interaction of adrenergic agents with...


