
Imidazole administered intraperitoneally to albino rabbits at a dose of 250 mg. per kilogram inhibited the rise of aqueous humor protein concentration by approximately 50 per cent 30 minutes after paracentesis. Systemic imidazole administered daily to albino rabbits injected with intravitreal Shigella endotoxin decreased the conjunctival and iris hyperemia and reduced the anterior chamber cell and flare and the haziness of the optical media. Systemically administered imidazole had no effect on the aqueous humor concentrations of c-AMP or c-GMP in the rabbit. In vitro studies of rabbit ciliary body-iris phosphodiesterase activity indicated no effect of imidazole at a concentration of 10^-3 molar.

In previous papers we have reported that imidazole administered intravenously or intraperitoneally to rabbits blocks the rise in intraocular pressure (IOP) and aqueous humor protein concentration following topical application of prostaglandin E, (PGE, to the eye.) The present studies were undertaken to determine the effect of imidazole on two other challenges to the integrity of the blood-aqueous barrier of the rabbit eye, paracentesis and intravitreal injection of Shigella endotoxin. In addition, information was sought on the effects of imidazole on the aqueous humor concentrations of cyclic adenosine monophosphate (c-AMP) and cyclic guanosine monophosphate (c-GMP), and on ciliary body-iris phosphodiesterase (PDE) activity.

Materials and methods.

Paracentesis. Adult albino rabbits 2 to 3 kg. in weight were lightly anesthetized by intravenous pentobarbital. Seven rabbits were pretreated with imidazole, 250 mg./kg. intraperitoneally, 4 hours prior to the initial paracentesis. Seven rabbits received intraperitoneal saline. Aqueous humor, 0.1 ml., was aspirated with a 27-gauge needle. Thirty minutes later the anterior chamber was emptied with a 27-gauge needle. The protein concentration of the primary and secondary aqueous humor samples was determined by the method of Lowry and co-workers. Shigella endotoxin. Shigella endotoxin (Difco Laboratories, Detroit, Mich.) was diluted with saline and passed through a Millipore filter. Endotoxin, 10 μg in a total volume of 50 μl, was injected through the pars plana into the center of the vitreous humor in both eyes of eight rabbits. Four rabbits received imidazole, 250 mg./kg. intraperitoneally, each day for the duration of the study. The other four rabbits received saline. The rabbits were examined daily with a Haag-Streit slit lamp and an ophthalmoscope and graded on an arbitrary scale of 0 to 4+ as to conjunctival and iris hyperemia, anterior chamber cell and flare, and haziness of the optical media. In grading the hyperemia or the anterior chamber reaction, the following system was used: 0, no detectable reaction upon slit-lamp observations; 1+, mild reaction; 2+, moderate reaction; 3+, marked reaction; and 4+, severe reaction. In grading the haziness of the optical media, the following system was used: 0, a 20/20 view of the retina upon examination with a direct ophthalmoscope; 1+, a 20/25 to 20/80 view; 2+, a 20/100 to barely detectable view; 3+, a red reflex only; and 4+, no red reflex.
Cyclic nucleotides. Eight albino rabbits received imidazole, 250 mg./kg. intraperitoneally. Eight rabbits received intraperitoneal saline and served as controls. Four hours later aqueous humor was aspirated with a 27-gauge needle. Aqueous humor concentration of c-AMP was determined by the technique of Harper and Brooker and the concentration determined by the commercially available radioimmunoassay kit from Schwarz/Mann Division, Becton Dickinson & Co. (Orangeburg, N. J.). The c-GMP was acetylated by the technique previously described. Unlabelled c-AMP was added to 0.50 /uCi of 1.25 H-c-AMP (40 Ci./mM) containing 5 mM MgCl2 were added; the tissue was homogenized and then sonicated for 15 seconds with a Sonic Dismembrator (Artek Systems Corp., Farmingdale, N. Y.) at a setting of 60. The homogenate was spun for 15 minutes at 450 x g at 4° C. The supernatant was retained at 4° C. and used within 72 hours. No loss of activity was noted during 3 days in these or other experiments.

PDE activities were determined by a technique previously described. Unlabelled c-AMP was added to 0.50 /uCi of 1.25 H-c-AMP (40 Ci. per millimole) to yield five final concentrations ranging from 0.22 to 3.12 /uM for the study of the low K m c-AMP PDE. Five concentrations of c-AMP ranging from 5 to 80 /uM were employed for the study of the high K m c-AMP PDE. Unlabelled c-GMP was added to 0.25 /uCi of 2.50 /uM H-c-GMP (10 Ci./mMol.) to yield five final concentrations of 5 to 40 /uM for the study of the c-GMP PDE.

Assays were conducted in triplicate at each substrate concentration in the presence and absence of 10-6 M imidazole. The reaction was stopped by boiling for 2 minutes. Snake venom, 5'-nucleotidase (Sigma Chemical Co., St. Louis, Mo.), was added to the mixture to convert the 5'-AMP or 5'-GMP to adenosine or guanosine. The residual cyclic nucleotide was adsorbed on AG 1-X2 (Bio-Rad Laboratories, Richmond, Calif.) resin and removed. The amount of adenosine or guanosine produced was then determined by scintillation counting. Results were corrected for boiled homogenate controls.

Results. Paracentesis. Pretreatment with systemic imidazole inhibited the increase in aqueous humor protein concentration following paracentesis by approximately 50 per cent (Table I).

Shigella endotoxin. During the 9 days of observation following intravitreal injection of Shigella endotoxin, a severe uveitis developed and then subsided. The rabbits treated with daily injections of imidazole demonstrated decreased conjunctival and iris hyperemia, less anterior chamber cell and flare, and reduced haziness of the optical media (Fig. 1).

Cyclic nucleotides. Intraperitoneal injections of imidazole did not alter aqueous humor concentrations of c-AMP or c-GMP (Table II).

Phosphodiesterase. Imidazole at a concentration of 10-4 molar had no effect at any of the five substrate concentrations studied on ciliary body—iris low k m c-AMP PDE, high k m c-AMP PDE, or c-GMP PDE. No influence of imidazole on the maximum velocities or the Michaelis constants characterizing the three enzyme systems was noted (Table III).

Discussion. Systemic imidazole is capable of...
Fig. 1. Ocular reaction to intravitreal injection of *Shigella* endotoxin. •, Saline-treated rabbits (8 eyes); □, imidazole-treated rabbits (8 eyes). *Statistically significant difference between the means of the imidazole-treated eyes and the saline-treated eyes, p <0.05 (Student t test).

blocking the rise in IOP and aqueous humor protein concentration following topical application of PGE$_2$ to the eye. The present studies indicate systemic imidazole is also capable of stabilizing the blood-aqueous barrier to paracentesis and intravitreal injection of *Shigella* endotoxin. Bengtsson" reports that intraperitoneal imidazole blocks the aqueous flare response in rabbit eyes induced by topical PGE$_2$, topical arachidonic acid, infrared irradiation of the iris, intravenous *Proteus* endotoxin, and subcutaneous α-melanocyte-stimulating hormone. Neither of these reactions is thought to be mediated by prostaglandins. Our studies do not rule out the possibility that phosphodiesterase activity is stimulated or cyclic nucleotide concentrations are lowered by imidazole in other ocular tissues or in isolated portions of ocular tissues important in the inflammatory response of the eye.

Many of the inflammatory responses of the eye are thought to be mediated by prostaglandins. It is known that PGE$_2$ can activate ciliary process adenylate cyclase. Butcher and Sutherland report that imidazole increases the activity of c-AMP PDE. Thus, the anti-inflammatory activity of imidazole could be the result of a decrease in ocular c-AMP caused by a stimulation of c-AMP PDE activity.

Present studies indicate that systemic imidazole in a dose known to have anti-inflammatory activity has no effect on aqueous humor concentrations of c-AMP or c-GMP or on ciliary body-iris PDE activity. It thus seems unlikely that the anti-inflammatory properties of imidazole are related to a stimulation of PDE in the rabbit eye. Furthermore, it is known that systemic imidazole is capable of blocking the IOP rise produced by topical nitrogen mustard and the aqueous flare reaction produced by subcutaneous α-melanocyte-stimulating hormone. Neither of these reactions is thought to be mediated by prostaglandins.

Our studies do not rule out the possibility that phosphodiesterase activity is stimulated or cyclic nucleotide concentrations are lowered by imidazole in other ocular tissues or in isolated portions of ocular tissues important in the inflammatory response of the eye.

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Key words: imidazole, paracentesis, *Shigella* endotoxin, c-AMP, c-GMP, phosphodiesterase.

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Mechanism of steroid action in ocular inflammation: Inhibition of prostaglandin production. Nava Floman and U. Zor.

Prostaglandin E (PGE) concentration in the aqueous humor of an intact rabbit eye was less than 0.1 ng per milliliter and increased to 19 ± 3 ng per milliliter 60 minutes following paracentesis. The rise in PGE level was associated with clinical signs of ocular inflammation. Pretreatment with triamcinolone reduced both the accumulation of PGE in the aqueous humor and the inflammatory response following paracentesis. Intravitreal injection of E. coli endotoxin into rabbit eyes increased PGE level in the anterior chamber to 72 ± 17 ng. per milliliter and induced acute uveitis. Slices of iris and ciliary body (ICB) derived from either rabbit eyes with endotoxin-induced uveitis or normal eyes were incubated for 60 to 240 minutes and the rate of PGE release from inflamed ICB was threefold higher than that of normal ICB. Incubation of inflamed ICB with hydrocortisone, or Millicorten (100 ng per milliliter) for 4 hours reduced PGE accumulation in the medium by 50 and 81 per cent, respectively. Aldosterone had no effect on the rate of PGE release from inflamed ICB throughout the incubation period. Hydrocortisone or Millicorten also reduced PGE tissue content of inflamed ICB by about 74 per cent during the period of incubation. Indomethacin (100 μg per milliliter) abolished PGE accumulation. The suppressive action of hydrocortisone on PGE release into the incubation medium was prevented by the addition of arachidonic acid (2 μg per milliliter), a substrate for prostaglandin synthesis. By contrast, the inhibitory action of indomethacin was not affected by provision of arachidonic acid. We suggest that glucocorticosteroids reduce PGE accumulation by limiting the availability of the substrate for prostaglandin biosynthesis and thus suppress the inflammatory response.

Prostaglandins (PG's) are regarded as mediators of the inflammatory process in many organs, including the eye.2,3 High levels of PG's have been demonstrated in aqueous humor of human and laboratory animals in some forms of ocular inflammation.4 Administration of exogenous PG's induces the characteristic signs of ocular inflammation.5 The involvement of PG's in the inflammatory response has been emphasized by the recent discovery that the anti-inflammatory properties of aspirin-like drugs are related to their direct inhibitory action on the microsomal PG synthetase system.6

In contrast to aspirin-like drugs, corticosteroids (CS) are devoid of a direct inhibitory action on PC synthetase activity in microsomal fractions. However, this does not exclude the possibility that the anti-inflammatory properties of CS are related to suppression of over-all PG synthesis and/or release from intact tissue. Indeed, several reports suggesting an inhibitory action of CS on PG synthesis and release were recently published.7,8 The present investigation was undertaken to explore whether CS interfere with: (1) the in vivo prostaglandin E (PGE) release into the anterior chamber following paracentesis of rabbit eyes and (2) the in vitro PGE synthesis and release by slices of inflamed rabbit iris and ciliary body (ICB). Preliminary results have been presented.9

Method and Materials.

In vivo studies. Adult albino rabbits weighing 2.5 to 3.5 kilograms were anesthetized with 50 mg. of Ketalar and 30 mg of Nembutal per kilogram of body weight, administered simultaneously intramuscularly. The right eye was treated with 0.1 ml. of triamcinolone acetonide (40 mg per milliliter) injected subconjunctivally. One hour later ocular inflammation was induced in both eyes by an anterior chamber paracentesis procedure previously described by Miller, Eakins, and Atwal.4 Sixty minutes following paracentesis, samples of aqueous humor (to be known as "secondary" aqueous) were withdrawn from both eyes with a 27 gauge needle. To facilitate the removal of fluid with a high protein content from the anterior chamber, the animals were heparinized.

Monocular uveitis in anesthetized rabbits was induced by intravitreal injection, at the pars plana, of 10 μg of E. coli endotoxin in 0.05 ml. of saline with a 27 gauge needle. Then 50 μl of saline were injected in the same way into the contralateral eyes, which served as controls. Thirty-six hours after injection of endotoxin the animals were...