ACTIONS AND INTERACTIONS OF BRADYKININ, PROSTAGLANDINS, AND NONSTEROIDAL ANTIINFLAMMATORY AGENTS ON THE EYE

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Abstract—Intracameral injections of bradykinin into the eyes of rabbits anesthetized with urethane were found to produce a dose-dependent constriction of the pupil and increase in the amount of protein present in the aqueous humor. Both these effects were relatively fast in onset, pupillary constriction being observed 1–2 min after the injection. The intact bradykinin molecule was required to produce these effects since prior incubation of known amounts of bradykinin with chymotrypsin and subsequent intracameral injection were without effect. No kininase activity was observed in samples of normal aqueous humor, however, kininase activity was present in aqueous humor removed from eyes inflamed by either paracentesis or nitrogen mustard. The actions of bradykinin on both the pupil and the protein content of aqueous humor were markedly reduced or abolished by pretreatment with inhibitors of prostaglandin biosynthesis, such as indomethacin or piroprofen, given either topically or by intraperitoneal injection. In these animals the simultaneous injection of prostaglandin E2 together with the bradykinin restored the ocular responses to normal. These results suggest that prostaglandins contribute to the ocular actions of bradykinin.

INTRODUCTION

It is now well established that the eye responds to irritation and trauma with a characteristic sequence of events involving pupillary constriction (miosis), local ocular vasodilation, and increased capillary permeability manifested as a breakdown of the blood–aqueous barrier leading to an increase in the protein content of the aqueous humor. It has been proposed that endogenous mediators such as prostaglandins and kinins may be responsible for some of

1 This work was supported in part by USPHS grants EY 00457 and EY 00091 and USPHS Training Grant GM 00438.
these events (1). Prostaglandins can reproduce the characteristic signs of acute inflammation in the eyes of experimental animals, and prostaglandins have been demonstrated in aqueous humor during some, but not all, forms of ocular inflammation (2). However, there have been few studies on the ocular actions of bradykinin (3). The present experiments were undertaken to determine the effects of nonsteroidal antiinflammatory drugs on the ocular actions of bradykinin in order to assess its possible role in certain forms of ocular inflammation.

MATERIALS AND METHODS

New Zealand white rabbits weighing 2-3.5 kg were anesthetized with 1-2 g/kg urethane injected into a marginal ear vein as a 25% solution in 0.9% NaCl solution. One 27-gauge needle was then introduced through the cornea into the anterior chamber of each eye, great care being taken to ensure that the needle did not touch the iris at any time during the experiment. Each needle was connected via polyethylene tubing to a calibrated Agla (Burroughs Wellcome) micrometer syringe for the intracameral injections. All such injections were made simultaneously into both eyes, and in all cases a standard volume of 10 μl was used.

Measurement of Pupil Diameter. The pupil size was measured visually in uniform artificial illumination with a transparent plastic millimeter ruler across the horizontal meridian. Two readings were made at -5 min, 0 time, and 5, 10, and 15 min after the intracameral injection.

Measurement of Aqueous Humor Protein. Samples of aqueous humor were removed from the anterior chambers of both eyes 15 min after the intracameral injection. Again, great care was taken to ensure that the needle of the syringe into which the aqueous was withdrawn did not touch the iris. Quantitative colorimetric determinations of the protein in these samples were made by the method of Lowry et al. (4).

Assay for Kininase Activity in Aqueous Humor. 0.05 ml of aqueous humor were incubated with bradykinin in 0.05 M Tris buffer (pH 8.0) at 37°C at 0 time and 30, 60, and 120 min. Samples were boiled for 10 min and the contents diluted 1:10 with physiological saline and assayed on the isolated rat uterus preparation (5) to determine the quantity of bradykinin remaining.

Drug Solutions. Stock solutions of bradykinin (1 mg/ml) in 10⁻³ M p-toluene sulfonic acid were kept refrigerated (-10°C) and diluted immediately before use with Tris buffer (pH 7.4). Stock solutions of prostaglandins (10 mg/ml) were stored at -10°C in 95% ethyl alcohol and diluted to the desired concentration in Tris buffer (pH 7.4) as required.

Drugs Used. Prostaglandin E₂ (Upjohn Company), bradykinin triacetate (Calbiochem), indomethacin (Merck), and piroprofen (Ciba-Geigy).

RESULTS

Injections of bradykinin directly into the anterior chamber were found to produce a dose-dependent constriction of the pupil and increase in the amount of protein present in the aqueous humor. This was true provided