Antianaphylactic effects of dipivalyl epinephrine and related compounds in rat conjunctiva. Tadashi Iso, Kozo Uda, Hideyasu Yamachi, Nobuko Nakajima, and Hiroshi Suda.

Topically applied dipivalyl epinephrine (DPE) and related compounds have been found to inhibit passive anaphylactic reaction in rat conjunctiva. The order of activity is as follows: isoproterenol > DPE > epinephrine > norepinephrine. The antianaphylactic effect of DPE was antagonized by propranolol but was not affected by phentolamine. The effects of epinephrine, norepinephrine, and isoproterenol were also antagonized by propranolol but potentiated by phentolamine. From these findings, it was suggested that DPE not only exerts its antianaphylactic action through activation of β-adrenergic receptor but also itself has a little different action from epinephrine.

It is generally accepted that β-adrenergic stimulating agents have been shown to inhibit immediate hypersensitivity in various preparations. We have also demonstrated that topically applied epinephrine elicited inhibition of passive anaphylactic reaction in rat conjunctiva. Recently, dipivalyl epinephrine (DPE), a prodrug of epinephrine, was proposed for use in the treatment of glaucoma. This compound has been reported to penetrate rapidly various ocular tissues and to be hydrolyzed easily to epinephrine by the esterase found in ocular tissues. Much of the activity of DPE is clearly related to its metabolism to catecholamine. On the other hand, it is also reported that DPE itself has the ability to bind to β-adrenergic receptor in rabbit iris-ciliary bodies, but not to α-adrenergic receptor and that this activity is not due to breakdown of the prodrug to epinephrine. The present study was performed to examine effects of DPE and related compounds on passive anaphylactic reaction in rat conjunctiva, and the mode of action of DPE was evaluated.

Materials and methods

Animals. Male Wistar rats weighing 250 to 270 gm were used.

Antiserum. Rat antiserum to ovalbumin (chicken egg, grade V, Sigma Chemical Co., St. Louis, Mo.) was obtained according to the method of Mota. Rats were immunized with ovalbumin (1 mg/animal, intramuscularly) and Bordetella pertussis vaccine (1 ml/animal, intraperitoneally; Takeda Chemical Industries). Serum collected from each animal 12 days after immunization was pooled and frozen until use. The antibody titer of this serum was about 1:32 as estimated by the 72 hr passive cutaneous anaphylaxis test.

Passive anaphylactic reaction in rat conjunctiva. The diluted antiserum (1:4) in a volume of 0.05 ml was injected into the right bulbar conjunctiva of rats. The dose of diluted normal rat serum was similarly injected into contralateral conjunctiva. At 3 days later, the animals were challenged intravenously with ovalbumin solution (25 mg/kg) containing Evans blue (12.5 mg/kg). The animals were sacrificed 30 min after challenge, and leaked dye in the bulbar and palpebral conjunctiva was extracted by the method described previously. Removed eyelids and eyeball were immersed in the extracting solution composed of acetone and 0.5% sodium sulfate (7:3, v/v) for 48 hr at room temperature. Amount of dye in the extracted solution was determined spectrophotometrically at 620 nm.

Drugs. Drugs such as dl-DPE HCl (Allergan Pharmaceuticals, Irvine, Calif.), l-epinephrine bitartrate (Sigma), l-norepinephrine bitartrate (Sigma), and l-isoproterenol bitartrate (Sigma) were dissolved in physiological saline and adjusted to pH 7.0 with 0.1N NaOH. The solution in a volume of 10 µl was applied topically to both eyes 15 min before, just prior to, and 15 min after challenge.
The adrenergic receptor blocking agents phentolamine mesylate (Regitin; Ciba Pharm. Co., Summit, N. J.) and dl-propranolol HCl (Sigma) were applied 25 min and 10 min before challenge.

Results. Amount of leaked dye in the tissue was estimated as a parameter of the increased vascular permeability during passive anaphylactic reaction in rat conjunctiva. In the present experimental condition, amount of leaked dye in the antiserum-treated eye tissue was 13.9 ± 0.8 μg (n = 42), but no leaked dye was detected in the normal serum-treated tissue.

Topical application of DPE at a concentration of 0.1% or more elicited significant inhibition of dye leakage in a dose-dependent manner, although 100% inhibition was not obtained (Fig. 1). Similar inhibitory effects were produced by treatment with adrenergic stimulating agents such as isoproterenol, epinephrine, and norepinephrine, whereas adrenergic blocking agents such as phentolamine and propranolol had no direct effect on the reaction. The dose-response curves revealed that the activity of DPE was about five and 15 times those of epinephrine and norepinephrine, respectively, and four times weaker than that of isoproterenol. Table I shows influences of adrenergic blocking agents on the antianaphylactic action of DPE and related compounds. Propranolol (1%), a β-adrenergic blocking agent, reduced the antianaphylactic effects of 0.3% DPE, 0.1% isoproterenol, 1% epinephrine, and 3% norepinephrine. Phentolamine (1%), an α-adrenergic blocking agent, enhanced the effects of 0.05% isoproterenol, 1% epinephrine, and 1% norepinephrine but did not alter that of 0.1% DPE.

Discussion. The present study revealed that β-adrenergic stimulating agents produced the inhibitory effect on passive anaphylactic reaction in rat conjunctiva and that this effect followed their order of activity as β-adrenergic stimulants (isoproterenol > epinephrine > norepinephrine). Moreover, it was demonstrated that the antianaphylactic effects of β-adrenergic stimulants were antagonized by propranolol but potentiated by phentolamine. These findings support the notion that β-adrenergic receptors are involved in the inhibitory effects of β-adrenergic stimulants on various types of experimental anaphylaxis. 1, 2 In addition, it is indicated that α-adrenergic receptors may be associated with β-adrenergic receptors in the present experimental anaphylaxis model.

On the other hand, DPE also produced marked inhibition of the passive anaphylactic reaction in a dose-dependent manner, and the activity was about five times that of epinephrine. The inhibitory effect of the prodrug, similar to that of epinephrine, was antagonized by propranolol. Thus these data may be in accordance with a general acceptance that after its metabolism to epinephrine, DPE acts like epinephrine. However, since the effect of DPE was not potentiated by phentolamine, it is indicated that DPE acts on the conjunctiva through a different mechanism from that of epinephrine. A possible assumption is that absorbed DPE is not all converted to epinephrine in the tissue and that unchanged DPE exerts its own pharmacological action. In fact, it is reported that DPE itself is active at a β-adrenergic receptor in isolated rabbit iris-ciliary bodies but not at an α-adrenergic receptor. 6 Thus it would seem reasonable to assume that DPE itself has no α-adrenergic activity in the conjunctiva, unlike all the other adrenergic agonists, and exerts its antianaphylactic action through activation of β-adrenergic receptor.

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Lysosomal hydrolases in tears and the lacrimal gland: effect of acetylsalicylic acid on the release from the lacrimal gland.

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Free and lysosomal activities of 10 acid hydrolases have been determined in human lacrimal gland tissue. The enzyme activities appeared mainly in the lysosomal fraction, and the proportions correspond very well with those found in the tear fluid. Acetylsalicylic acid at blood levels of about 1 mM produced a significant lowering of the β-hexosaminidase concentration in tears. It is suggested that acetylsalicylic acid may have a stabilizing effect on lacrimal lysosomes, resulting in a diminished release of lysosomal enzyme from the lacrimal gland.

Lysosomal hydrolases are present in human tears, and the main source of these enzymes is to be found in the lacrimal gland. This was demonstrated in assays of samples of tear fluid obtained by nonmechanical stimulation with tear gas. The conjunctival epithelium may act as a second source, but this is only noticeable if tear samples are obtained under slight epithelial damage with filter paper strips. This paper presents further evidence for the origin of lacrimal lysosomal hydrolases and the effect of acetylsalicylic acid as lysosomal stabilizer in vivo.

Materials and methods. Human lacrimal gland tissue was obtained occasionally from orbital operations in the University Eye Clinic, Amsterdam.

Tear fluid was obtained from normal volunteers after stimulation with vapor of 2-chloracetophenone and collected in glass capillaries, discarding the first 10 μl. The next specimens—minimally 20 μl of tear fluid—could be regarded as lacrimal gland fluid in which admixture with conjunctival secretions was reduced to the minimum.

Tissue homogenates were prepared with 0.25M sucrose, pH 7.4, in a Potter-Elvehjem homogenizer with a Teflon pestle. The whole homogenate was centrifuged at 1000 × g for 5 min. The pellet was resuspended in 0.25M sucrose, pH 7.4, and recentrifuged at 1000 × g for 5 min. The pellet was then treated with 0.1% Triton X-100 and centrifuged at 10000 × g for 10 min. The Triton X-100 supernatant was used for the estimation of the amount of enzymes discarded with the debris. In all experiments the enzyme activity in the debris was less than 5% of the total activity. The combined sucrose supernatants were centrifuged at 50,000 × g for 30 min. The pellet was treated with 0.1% Triton X-100, and this extract was used for the determination of the lysosomal fraction.

Latency of the glycosidase activities was determined in a 50,000 g pel under conditions in which lysosomes had appeared to be stable. In a 15 min incubation at 37° C and at pH 5.2 in 0.25M sucrose, free activities could be assayed without measurable breakdown of lysosomes. The total activities were measured under the same conditions in the presence of 0.1% Triton X-100.

The 50,000 g supernatant was split into two parts, and these were dialyzed against, respectively, 0.05M acetate and citrate-phosphate bufl-