Electrolytes in lacrimal gland fluid and in tears at various flow rates in the rabbit

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HYPOTHESES about the mechanism of secretion of electrolytes by the lacrimal gland are based on electrophysiological studies (7, 10, 19) and studies in which the concentration of electrolytes of conjunctival fluid, i.e., tears, has been determined (10, 18, 19). Because tears in the conjunctival sac consist of a mixture of fluids secreted by all the orbital glands, it is important to know if conclusions about secretory mechanisms of the lacrimal gland based on analyses of tears are valid. The present study was undertaken to determine the relative contribution of the fluid collected directly from the excretory duct of the lacrimal gland, i.e., the lacrimal gland fluid, to the conjunctival fluid and to gain information about how the lacrimal gland secretes electrolytes by comparing, at various flow rates, the concentration of electrolytes in lacrimal gland fluid, uncontaminated by fluid from the other orbital glands, such as the Harderian, Nictitans, and conjunctival in the rabbit.

MATERIAL AND METHOD

All experiments were done in an illuminated room on white male New Zealand rabbits weighing 3.31 ± 0.07 kg (mean ± se; n = 35), anesthetized with sodium pentobarbital (initial dose, 40 mg/kg) and artificially ventilated through a tracheal cannula. Blood pressure was continuously monitored from a cannula placed in a femoral artery. Timed samples of lacrimal gland fluid were collected on preweighed pledges of plastic sponge (Week-cel, Edward Week & Co., Inc.; dry weight = 0.02555 ± 0.00183 g; n = 35), each of which was placed, at varying intervals, at the distal ends of a 4.3 ± 0.4 mm (n = 5) long P-10 polyethylene cannula, which had been inserted 2.1 ± 0.09 mm (n = 5) into the excretory duct of the lacrimal gland and left in place throughout the experiment. We would like to point out that the lacrimal gland in the rabbit has been described as having two ducts, which open into the conjunctival sac (5). Therefore, in the first 150 rabbits, in which the excretory duct was cannulated in our laboratory, the conjunctiva was carefully observed with a dissecting microscope. An opening was located near the lateral canthus on the inferior lid in every rabbit. Retrograde injection of a dye into this opening always resulted in staining of all portions of the lacrimal gland. In only six (4%) rabbits were there two openings, and when the smaller orifice was obstructed, neither flow of conjunctival fluid nor flow from the cannula in the larger orifice was changed. For these reasons, we believe that although in those few cases when only one of two ducts is cannulated, fluid from the major portion, if not all, of the lacrimal gland is collected. A second duct could account for a small part of the variation in flow rates which we observed, but the presence of a second duct in a few rabbits would not alter the conclusions based on the statistical analysis of our data. Timed samples of tears, i.e., conjunctival fluid resulting from secretion of all the orbital glands, including the lacrimal, were collected on preweighed pledges (dry weight, 0.03187 ± 0.00193 g) from a conjunctival trough, which was made in the supine rabbit by slightly stretching the superior lid with an appropriately placed weight. Samples plus sponge were weighed and diluted with 5.0 ml deionized water approximately 1 min after the end of the collection period. Diluted samples were placed in a cold room (4°C) and, on the following day, were analyzed for chloride with an Amino-Cotlove titrator and for sodium and potassium by flame emission spectrophotometry. Concentrations were corrected for the amount of electrolytes in the diluting fluid (mEq/liter: Na+ = 0.0008 ± 0.0005, K+ = 0.0019 ± 0.0002; Cl− = 0.054 ± 0.005) and extracted from the sponge (mEq/liter per g: Na+ = 0.42 ± 0.01, K+ = 0.010 ± 0.002; Cl− = 0.05 ± 0.02). Sample volumes were calculated from the difference between wet and dry sponge weights (lacrimal: 0.02049 ± 0.00142 g with maximal and 0.00110 ± 0.00036 g with minimal flow; conjunctival:
gland fluid, and tears removed before the samples were collected. Various placed on the cornea and conjunctiva for 2 min and then

| Table 1. Data from analyses of lacrimal gland fluid col-

| TABLE 1. Electrolytes in artificial tears, lacrimal gland fluid, and tears |

<table>
<thead>
<tr>
<th>Sample Vol, µl</th>
<th>Collecting Time, min</th>
<th>Flow, µl/min</th>
<th>Concentration, mEq/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artificial tears</td>
<td>Na⁺ = 115 mEq/liter</td>
<td>1.2051</td>
<td>0.0701</td>
</tr>
<tr>
<td></td>
<td>K⁺ = 15 mEq/liter</td>
<td>97.7995</td>
<td>1.89</td>
</tr>
<tr>
<td></td>
<td>Cl⁻ = 120 mEq/liter</td>
<td>1.0171</td>
<td>0.0600</td>
</tr>
<tr>
<td>Lacrimal gland fluid</td>
<td>Min basal flow</td>
<td>1.131</td>
<td>16.38</td>
</tr>
<tr>
<td></td>
<td>Max stim flow</td>
<td>27.1577</td>
<td>1.93</td>
</tr>
<tr>
<td>Tears</td>
<td>Min basal flow</td>
<td>1.2900</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Max stim flow</td>
<td>23.1836</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Values are means ± SE. Numbers in parentheses = number of rabbits. Corrected for evaporation rate (0.036 ± 0.002 µl/min in all groups; see text). Collecting time does not include diluting time (1.0 ± 0.08 min in all groups, see text). Basal = cornea and conjunctiva anesthetized with proparacaine.

0.02456 ± 0.00312 g with maximal and 0.00124 ± 0.00046 g with minimal flow). Volumes were determined by serial weighings of a volume of deionized water during each sampling period. Weighing was performed on a rapid-reset digital elapsed-time indicator, which read to 0.1 g with minimal flow). Volumes were corrected for evaporation rate (0.036 ± 0.002 µl/min), which was determined by serial weighings of a volume of deionized water during each sampling period. Weighing was performed on a rapid-reset digital elapsed-time indicator, which read to 0.1 g with minimal flow). Weighing and diluting times (60.1 ± 5.2 sec). Data from control analyses of 1- to 30-µl samples of an artificial tear solution, which were placed on precrushed pledgets of sponge with collecting, weighing, and diluting times similar to the lacrimal gland fluid samples and treated in the same manner as the lacrimal gland fluid samples, are given in Table 1. From data analyses of lacrimal gland fluid collected under oil in a closed system were comparable to data from analyses of lacrimal gland fluid collected by the open pledget system at similar slow flow rates (Table 2). To obtain basal flow rates, 50 µl of 0.5% proparacaine were placed on the cornea and conjunctiva for 2 min and then removed before the samples were collected. Various flow rates were obtained by reflex stimulation (i.e., spontaneously occurring flow in the absence of proparacaine) and by the intravenous administration of a cholinergic drug, pilocarpine HCl, in a dose of 0.4 mg/kg.

RESULTS

From the data in Table 1, it is apparent that: 1) the minimal basal flow rate for conjunctival fluid, obtained when the cornea and conjunctiva were anesthetized with proparacaine, was 10 times greater than the minimal basal flow rate for the lacrimal gland fluid collected under the same conditions. 2) The maximal flow rate of the conjunctival fluid was only twice the maximal flow rate of the lacrimal gland fluid. 3) The ratio of maximal flow rate/minimal flow rate for the lacrimal gland fluid was 5 times greater than that for the conjunctival fluid. 4) At maximal flow, the concentrations of sodium and the chloride in the lacrimal gland fluid were 3-4 times greater than that in the conjunctival fluid, but the concentration of potassium in the lacrimal gland fluid was the same as that in the conjunctival fluid. 5) At maximal flow, the concentration of sodium and chloride in the lacrimal gland fluid was the same as that in the conjunctival fluid, but the concentration of potassium in the lacrimal gland fluid was twice that in the conjunctival fluid.

With flow rates less than 0.5 µl/min, the sodium and chloride concentration in the lacrimal gland fluid decreased as the flow rate increased, but the potassium concentration in lacrimal gland fluid did not change with increasing flow rate (Fig. 1). With flow rates between 2 and 14 µl/min, the concentrations of sodium, chloride, and potassium in the lacrimal gland fluid did not change as the flow rate increased (Fig. 1). However, with flow rates between 2 and 30 µl/min, although there was a tendency for the concentration of potassium in tears to increase with an increase in flow rate, the concentration of sodium and chloride in tears did not change as flow rate increased (Fig. 1).

DISCUSSION

It is customary to classify the orbital glands into basic secretors, i.e., those which secrete in the absence of neurogenic stimuli, and reflex secretors, i.e., those, such as the lacrimal, which secrete in response to parasympathetic nerve impulses (2, 3, 6, 13) resulting from psychogenic stimuli or from reflexes produced by stimulation of the cornea, conjunctiva, nasal mucosa, and retina (9). The results of the present study, in which flow from the lacrimal excretrory duct and tear flow have been measured by the same technique, indicate that this classification is not accurate. Thus, our data demonstrate that the lacrimal gland secretes a significant amount, i.e., 1/10th the tear volume, under basal conditions, since it is unlikely that the minimal flow that we measured when the cornea and conjunctiva were anesthetized resulted from psychogenic tearing, which cannot be demonstrated in subhuman species (4), or from reflex tearing, which requires strong nasal or retinal stimuli (9). Furthermore, our results show that other orbital glands, in addition to the lacrimal, respond to neurogenic and pharmacological stimuli. Thus, with maximal flow the volume contributed to tears by other orbital glands was the same as that of the lacrimal, even though the increase in output by the lacrimal was 5 times that of the other orbital glands.

Before discussing the significance of our data with respect to the physiological function of the lacrimal glands, it is necessary to recognize that the lacrimal gland is not a simple gland, but a complex organ that secretes a complex fluid. The lacrimal gland is a gland that secretes a fluid that is highly specialized for the protection of the cornea and conjunctiva. The lacrimal gland secretes a fluid that is rich in proteins, particularly immunoglobulins, which help to keep the cornea and conjunctiva healthy. The lacrimal gland also secretes a fluid that is rich in salts, which help to keep the cornea and conjunctiva moist. The lacrimal gland also secretes a fluid that is rich in enzymes, which help to keep the cornea and conjunctiva healthy.

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to the concentrations of electrolytes at various flow rates, we would like to point out that it is unlikely that the high electrolyte concentrations found in this study were due entirely to evaporation because: 1) the concentration of Na⁺, Cl⁻, and K⁺ in 1- to 30-μl samples of an artificial tear solution, which had been delivered onto pledgets of plastic sponge with flow rates, collecting times, weighing, and diluting times similar to those encountered in these experiments, did not differ significantly from the expected values (Table 1). 2) The ratio of Na⁺ and of Cl⁻ to K⁺ in the lacrimal gland fluid did not remain constant but decreased as flow rate increased (Fig. 1). 3) Despite the fact that the evaporative surface for the tear samples was larger than that for the lacrimal gland fluid samples, at comparable flow rates, the K⁺ concentration was lower and the Na⁺ and Cl⁻ concentrations were the same in tears as in the lacrimal gland fluid. 4) Values for electrolyte concentrations were the same in samples obtained with the open pledget method as those obtained with a closed system with similar flow rates (Table 2). 5) Correcting for evaporation by using data from an in vitro system, i.e., the evaporation rate from a pure water surface, is open to criticism. However, Iwata, Lemp, Holly, and Dohlman (8) have shown that even when tear flow rate and fluid replacement are eliminated, the rate of evaporation from a pure water surface was faster than that from the rabbit cornea with and without an intact tear film.

As summarized by Schultz (17), micropuncture studies of other exocrine glands have demonstrated that the primary fluid secreted by the acinar cell is plasmalike with respect to the electrolyte concentration. If the primary fluid in the rabbit lacrimal gland is also similar to plasma, our measurements of the final lacrimal gland fluid as collected from the excretory duct indicate the following: at minimal basal, i.e., unstimulated, flow rates, water may be reabsorbed distal to the acinus, since at these flow rates the concentrations of these three electrolytes were higher than plasma levels. However, when the rabbit lacrimal gland was stimulated, we found that: 1) it secreted K⁺ distal to the acinus, as do salivary (11, 12, 14) and rat exorbital flow, i.e., with proparacaine local anesthesia and pilocarpine (triangles, base up). Also shown at various flow rates are mean values of 5 determinations of plasma electrolyte concentrations and ratios of Na⁺/K⁺ (circles) and Cl⁻/K⁺ (crosses).

**Table 2. Lacrimal gland fluid electrolytes**

<table>
<thead>
<tr>
<th>Flow, μl/min</th>
<th>Open Method* (9)</th>
<th>Closed Method (8)</th>
<th>Open Method* (9)</th>
<th>Closed Method (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Na⁺], mEq/liter</td>
<td>396±19</td>
<td>370±15</td>
<td>32±4</td>
<td>27±3</td>
</tr>
<tr>
<td>[K⁺], mEq/liter</td>
<td>519±122</td>
<td>500±22</td>
<td>240±36</td>
<td>273±32</td>
</tr>
<tr>
<td>[Cl⁻], mEq/liter</td>
<td>396±430</td>
<td>370±15</td>
<td>227±32</td>
<td>264±46</td>
</tr>
</tbody>
</table>

Values are means ± se. Numbers in parentheses = number of determinations. * Fluid collected on pledget of plastic sponge. Values corrected for evaporation rate (see METHODS for details).
LACRIMAL GLAND FLUID AND TEAR ELECTROLYTES

lacral (1) glands, since the K\(^+\) concentration increased as flow rate increased. 2) It neither reabsorbed nor secreted Na\(^+\) distal to the acinus, since the Na\(^+\) concentration remained constant over a wide range of stimulated flow rates. Thus, this gland apparently handles sodium as the pancreas (16) does but not as do those glands that reabsorb Na\(^+\) distal to the acinus, e.g., salivary (11, 12, 14), sweat (15), and rat exorbital lacrimal (1) glands. 3) It does not reabsorb or secrete Cl\(^-\), since the Cl\(^-\) concentration was constant over a wide range of stimulated flow rates, unlike those glands that do reabsorb Cl\(^-\), e.g., salivary (11, 12, 14), sweat (15), pancreas (16), and rat exorbital lacrimal (1) glands.

Our data are compatible with the hypothesis that in the unstimulated rabbit lacrimal gland the acinus secretes a plasmalike primary fluid and water is reabsorbed distally. When the gland is stimulated, the acinus continues to secrete a plasmalike primary fluid, but K\(^+\) is secreted between the acinus and the lower end of the excretory duct and the distal water reabsorption may be inhibited.

Because there are differences between the final lacrimal gland fluid and tears with respect to the electrolyte concentrations at various flow rates (Fig. 1), we believe that hypotheses about the mechanism of electrolyte secretion by individual orbital glands, such as the lacrimal, should not be based on data obtained from the analysis of tears, a term, which, in fact, should be used to designate fluid collected from the conjunctival sac composed of secretions from all the orbital glands.

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REFERENCES