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IONIC DEPENDENCE OF THE RESTING MEMBRANE POTENTIAL OF RABBIT LACRIMAL GLAND IN VITRO

CHUMPOL PHOLPRAMOOL and SUTAS KORPPAIBOOL

Department of Physiology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 4 (Thailand)

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Summary

1. Intracellular measurements of membrane potentials were made from acinar cells of rabbit lacrimal gland in vitro at 25 ± 0.3°C (± S.E.) by using 3 M KCl-filled glass microelectrodes.

2. The mean resting membrane potential in a Krebs-Ringer bicarbonate solution was −26.7 ± 0.3 mV, inside the cell negative.

3. Elevation of the external K^+ concentration ($[K^+]_0$) caused depolarization of the resting potential. When $[K^+]_0$ was above 5 mM, a tenfold increase in $[K^+]_0$ decreased the membrane potential by approx. 15 mV. This depolarization was not mediated by the release of acetylcholine (ACh) from the depolarized nerve terminals since it was seen in the presence of 10^{-5} M atropine.

4. Equimolar replacement of $[Na^+]_0$ by either choline or Tris at constant $[K^+]_0$ (5.8 mM) produced hyperpolarization. The resting potential was −33.1 ± 0.6 mV and −38.9 ± 0.6 mV, when 70 mM $[Na^+]_0$ was replaced by choline and Tris respectively.

5. The resting potential was not affected by either partial or complete substitution of $[Cl^-]_0$ with SO_4^{2-}.

6. Omission of Ca^{2+} from the external medium in the absence and in the presence of 5 mM EGTA decreased the membrane potential by 5.4 mV and 11.8 mV respectively.

7. It is concluded that the resting membrane potential of rabbit lacrimal gland is independent of the external Cl^- but it primarily depends on the external Na^+, K^+ and Ca^{2+}. The data were interpreted in terms of high membrane permeability to Na^+ and K^+ of the lacrimal gland cells. The relative permeability ratio, P_{Na}/P_K, in normal Krebs-Ringer bicarbonate solution was estimated to be 0.35.

Introduction

There have been only a few electrophysiological studies of lacrimal glands. Hisada and Botelho [1] found the resting membrane potential of the cat lacrimal

acinar cells, which could respond to lacrimal nerve stimulation, to be −28.9 ± 2 mV. On the other hand, Kikkawa [2] reported that the resting potential across the basal membrane of rabbit lacrimal gland in vivo was −37 ± 8 mV. However, these two studies made particular emphasis to the secretory potential in response to nerve stimulation [1] or to ACh administration [2]. In order to understand the ionic basis of secretory mechanism, it is essential to know the ionic permeability of the membrane at resting phase as well as secretory phase. The present study intends to elucidate this property of the membrane by investigating the effect of external ionic environments on the resting membrane potential of rabbit lacrimal glands in vitro. We used the in vitro preparations to avoid the technical difficulties in perfusing solutions of various ionic compositions in vivo.

Methods

Tissue preparations

Lacrimal glands were quickly removed from both male and female rabbits, which were anesthetized with intravenous sodium pentobarbital (35 mg/kg). The gland was decapsulated and was dropped into a petri dish containing Krebs-Ringer bicarbonate solution. A section of the gland was made by using a razor blade. A small segment weighing approximately 50 mg was then transferred to an agar platform in a perspex tissue bath (volume 1.5 ml). The segment was held in place by a cotton gauze pressed on top. The gauze was in turn secured to the platform by pins (Fig. 1). Normal Krebs-Ringer bicarbonate solution or modified solutions, which were continuously gassed with 95% O_2 and 5% CO_2, were superfused at room temperature (25 ± 0.3° C) by either gravity or by an infusion pump (Extracorporeal Medical Specialties, Model RD 074) at a rate of about 1 ml/min.

Membrane potential measurements

Membrane potentials were measured between a glass microelectrode, which was advanced into the gland cells by a hydraulic micromanipulator (Mechanical Developments Co.), and a reference electrode, which was a Ag/AgCl wire in an agar bridge (3% agar in 3 M KCl) placed in the tissue bath. The glass microelectrode was pulled from Kimax capillary tube (Kimax 46485, 0.7—1.0 mm outer diameter) by a horizontal micropipette puller (Industrial Science

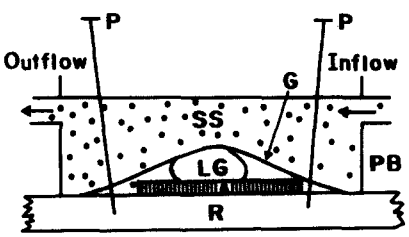

Fig. 1. Diagram showing lacrimal gland preparation superfused in a tissue bath. LG = segment of lacrimal gland, A = agar platform (3% agar in physiological saline), R = rubber ring, G = cotton gauze, P = pin, PB = perspex tissue bath, SS = superfusion solution.

Associates) to a tip diameter of less than 1 μm. The microelectrode was filled with 3 M KCl according to the method of Tasaki et al. [3]. Only the electrode with tip resistance of between 7 and 50 MΩ and tip potential of less than 5 mV was used in this study. The input from the microelectrode and reference electrode was displayed on an electrometer (Keithley Instruments, Model 610C) and simultaneously recorded on a type RM Beckman dynograph recorder through a DC preamplifier (Grass Instruments, Model P6-12).

In general, the tissue was allowed to equilibrate with incubating media for 20—30 min before membrane potential measurements were made. After such equilibration period resting potentials appeared to be steady for about 1—2 h. Only the impalement which showed the following characteristics was acceptable: (1) a sudden jump to peak voltage when the microelectrode penetrated into the cell [4]; (2) the maintenance of the potential for at least 1 min; (3) an abrupt return to the original baseline when the electrode was withdrawn from the cell. Typical recordings of the resting membrane potential of rabbit lacrimal gland are shown in Fig. 2. Since the resting membrane potential in the present study was similar to those observed in salivary gland cells of rats [5], the same criterion for the measurement of membrane potentials was employed, i.e. the maximum negativity after overshoot. It should be pointed out that not all the recordings shown in Fig. 2 were considered acceptable but only those which followed all criteria listed above, i.e., the 1st and 4th recordings from left, were measured in this study. All membrane potentials reported in this investigation had been corrected for the tip potential of the recording microelectrodes.

To investigate the ionic mechanism underlying the resting membrane potential, there are two approaches. One is to impale a cell, record the potential and, while recording, shift to a perfusate containing a different ionic concentration or composition. After a new steady potential is reached, the perfusate is shifted back to normal and the potential is followed until it regains its original value. The second approach is to record a series of control potentials from several cells, then switch to the test solution and record another series of resting potentials from different or if possible the same cell in the test solution, and finally back to the control solution. It has been shown that the results obtained from the two methods agreed well with each other in thyroid gland [6] and in brown adipose tissue [7]. The latter technique was also accepted and employed in several laboratories [8,9,10,11]. Since it was very difficult to maintain the microelectrode within a cell for longer than 1.5 min in the present investigation, the second approach was carried out. All data presented in this study are, therefore, the value obtained from different successfully impaled cells which are assumed to be samples of the same population. Nevertheless, the data were statistically treated as unpaired samples.

Solutions

Under normal conditions the gland was bathed in a Krebs-Ringer bicarbonate solution except during testing period when a modified solution was used. The composition of these solutions are shown in Table I. The solutions were prepared as described by Deluca and Cohen [12]. All solutions used were adjusted to pH 7.4 and the pH of the solutions were always checked before, during and after the experiments.

TABLE I

COMPOSITION OF KREBS-RINGER BICARBONATE AND ITS MODIFIED SOLUTIONS

All concentrations are in mM. All solutions contained in addition to stated composition 2.5 mM Ca^{2+} (except solution J), 1.2 mM Mg^{2+}, 1.2 mM $H_2PO_4^-$ and 5.6 mM Glucose. Each solution had the same pH, tonicity and ionic strength as the control medium except solution I and J in which the tonicity were 15 and 2% lower respectively, and the ionic strength was 4% greater for solution I and 4% lower for solution J.

Solution	Na^+	K^+	Cl^-	HCO_3^-	SO_4^{2-}	Choline	Tris/Cl	Tris base
Control (A)	140.6	5.8	125.7	24.5	1.2	—	—	—
K-free (B)	146.4	—	125.7	24.5	1.2	—	—	—
K-100 (C)	46.4	100	125.7	24.5	1.2	—	—	—
Na-free choline (D)	—	5.8	125.7	—	1.2	116.1	—	24.5
Na-free Tris (E)	—	5.8	125.7	—	1.2	—	116.1	24.5
Na-70-Tris (F)	70.0	5.8	125.7	12.3	1.2	—	58.1	12.3
K-50-Tris (G)	—	51.2	125.7	—	1.2	—	70.8	24.5
K-100-Tris (H)	—	101.2	125.7	—	1.2		20.8	24.5
Cl-free (I) *	141.0	5.8	—	24.5	59.5	—	—	—
Ca-free (J)	140.6	5.8	120.8	24.5	1.2	—	—	—

* This solution also contained 5 mM NO_3^-.

Results

The resting membrane potential during superfusion with normal Krebs-Ringer bicarbonate solution

Fig. 2 shows typical recordings of the resting membrane potential of rabbit lacrimal gland in seven successive impalements. The overall feature of these potentials is similar to that reported in rat salivary glands [5]. The resting potential obtained from 260 cells in 10 preparations ranged from −8 to −46 mV, inside the cell negative, with the mean value of −26.7 ± 0.3 mV. The frequency distribution of membrane potentials was unimodal with a slight skew to the left. Such potential profile was independent of the depth of microelectrode up to 400 μm from the gland surface. In spite of this fact, all subsequent data were obtained from cells at the depth of less than 300 μm from the surface.

It should be recalled that, in the following experiments, the effects of varying external ionic concentrations on the resting potential were determined

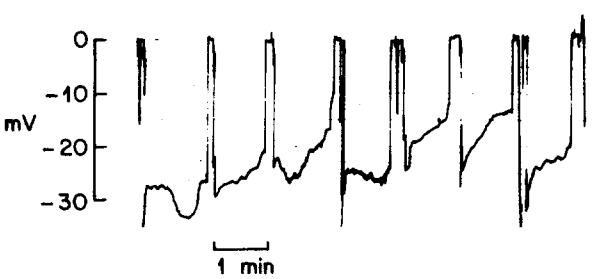

Fig. 2. A typical recording of the resting membrane potential of the rabbit lacrimal gland in seven consecutive penetrations. This series of impalements was made by a microelectrode with a resistance of 25 MΩ and tip potential of −5 mV.

by recording group of cells before, during and after changing the superfusion solutions.

Effects of varying $[K^+]_0$ at constant $[Na^+ + K^+]_0$

The concentration of K^+ in Krebs-Ringer bicarbonate solution was changed by replacement with equimolar Na^+ such that the concentration of $Na^+ + K^+$ was always 146.4 mM. Reduction of $[K^+]_0$ caused hyperpolarization of membrane potentials. On the other hand, depolarization was observed when $[K^+]_0$ was increased. The time courses of changes in membrane potentials after exposure to 20 and 100 mM $[K^+]_0$ are shown in Fig. 3. It appears that depolarization reaches the steady value 15—20 min after changing the superfusion solution. Hence, unless otherwise indicated, all later measurements were made after 15—20 min equilibration period. Fig. 4 summarizes the effects of various $[K^+]_0$ on the resting membrane potential. It is evident that, when $[K^+]_0$ is greater than 5 mM, a linear relationship holds between resting potential and log $[K^+]_0$ with a slope of approximately 15 mV per 10-fold changes in log $[K^+]_0$.

The elevation of $[K^+]_0$ in the superfusion fluid may release ACh from the depolarized nerve terminals [13]. Since it has been shown that ACh caused hyperpolarization of the acinar cell membrane potential of rabbit lacrimal gland [2], it might be possible that the direct depolarizing effect of K^+ was altered by the action of endogenous ACh. In order to test this possibility, atropine sulfate (10^{-5} M) was included in the superfusion fluid in the experiment in which the $[K^+]_0$ was 20 mM. Table II shows that depolarization of membrane potential in a solution containing 20 mM $[K^+]_0$ was not changed by 10^{-5} M atropine. Thus, the depolarization of the rabbit lacrimal gland is probably not affected by endogenous ACh, but it is occurred by the direct action of K^+.

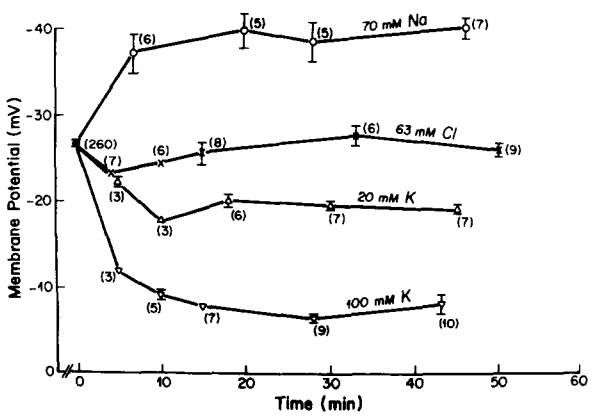

Fig. 3. Time-courses of the changes in the resting membrane potential by various external ionic concentrations. The Krebs-Ringer bicarbonate solution was replaced by the modified solutions containing 100 mM K^+ (▽), 20 mM K^+ (△), 63 mM Cl^- (X) and 70 mM Na^+ (○) at time zero. Each symbol represents mean ± S.E. (vertical line and bars) of a number of measurements which is indicated in each parenthesis.

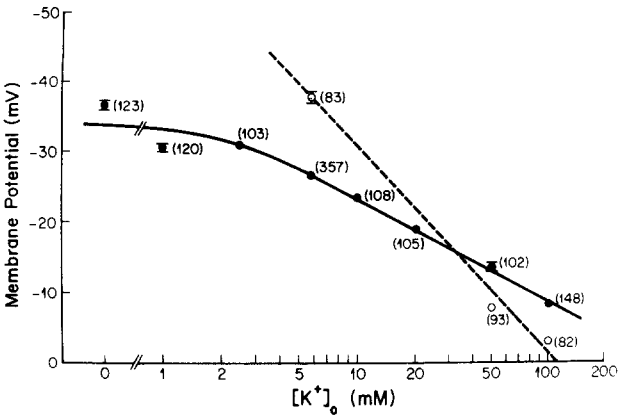

Fig. 4. Changes in the resting membrane potential as a function of external potassium concentrations. Closed circles and solid line are resting potentials in the media in which external potassium is replaced by sodium such that $[Na^+]_0 + [K^+]_0$ is always 146.4 mM. Open circles and broken line are resting potentials in the Na-free Tris solutions (solutions E, G and H in Table I). Each symbol represents mean ± S.E. (vertical line and bars) of a number of measurements which is indicated in each parenthesis.

Effects of varying $[Na^+]_0$ at constant $[K^+]_0$

When $[Na^+]_0$ was partially substituted by either choline or Tris (Tris-(hydroxymethyl)-aminomethane) while $[K^+]_0$ was kept constant as in control solution, i.e., 5.8 mM, membrane potentials were increased (hyperpolarized). Hyperpolarization occurred within 3 min and reached steady value by 15 to 20 min after exposure to the modified solution (Fig. 3). Fig. 5 shows the effects of reducing $[Na^+]_0$ at different concentrations on the resting potential. The maximum hyperpolarization appears to occur in the medium containing 35 mM $[Na^+]_0$ (Tris-for-Na replacement). Hyperpolarization decreased as $[Na^+]_0$ was further reduced. Thus, the resting potential was only −37.6 ± 0.7 mV when $[Na^+]_0$ was completely substituted by Tris. On the other hand, no significant hyperpolarization ($P > 0.2$) was observed when $[Na^+]_0$ was completely replaced by choline (Fig. 5). It should also be noted that substitution of $[Na^+]_0$ by Tris produced more hyperpolarizing effect than that by choline at all concentrations. The decrease in hyperpolarization at high concentrations of Tris and choline could, in part, be explained by their additional effects on the membrane permeability to other ions [7,14].

TABLE II

EFFECT OF 20 mM $[K^+]_0$ IN THE ABSENCE AND IN THE PRESENCE OF ATROPINE SULFATE (10^{-5} M) ON THE RESTING MEMBRANE POTENTIAL OF RABBIT LACRIMAL GLAND

Solutions	Membrane potential mean ± S.E. (mV)	Number of observations (n)	Level of significance for difference from the control *
Control	−26.5 ± 0.3	218	
20 mM K^+	−18.9 ± 0.3	105	$P < 0.001$
20 mM K^+ + 10^{-5} M atropine	−18.6 ± 0.4	82	$P < 0.001$, $P > 0.4$ **

* P values are from Student's t test for unpaired samples.

** Comparison between 20 mM K^+ and 20 mM K^+ + 10^{-5} M atropine solutions.

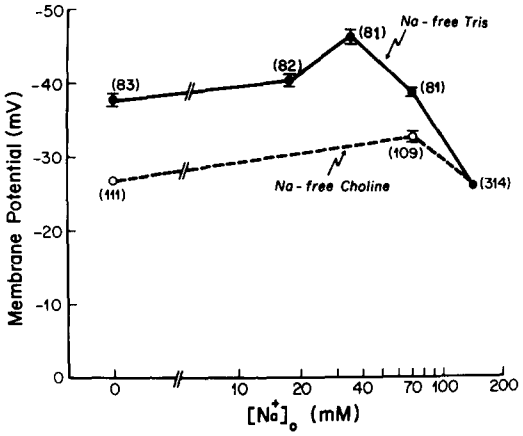

Fig. 5. Changes in the resting membrane potential as a function of external sodium concentrations. Closed circles and solid line are resting potentials in the solutions in which sodium is replaced by Tris. Open circles and broken line are resting potentials in the solutions in which sodium is replaced by choline. Each symbol represents mean ± S.E. (vertical line and bars) of a number of measurements which is indicated in each parenthesis.

Effects of varying $[K^+]_0$ in Na-free (Tris replacement) solution

As previous experiments showed that the resting potential of lacrimal gland was largely dependent on $[Na^+]_0$. It is likely that the effect of varying $[K^+]_0$ at constant $[Na^+ + K^+]_0$ may be complicated by the effect of changing $[Na^+]_0$ as well. Another series of experiments was performed to clarify this problem by varying $[K^+]_0$ in the Na-free media (solutions G, H). It was found that resting potentials were markedly depolarized by increasing $[K^+]_0$ in Na-free medium. The change in membrane potential per tenfold change in log $[K^+]_0$ was approximately 29 mV (Fig. 4). These results conform to the foregoing data that $[Na^+]_0$ make significant contribution to the resting potential in rabbit lacrimal gland.

Effects of varying $[Cl^-]_0$ at constant $[K^+]_0$ and $[Na^+]_0$

In order to determine whether Cl^- has any significant role on the resting potential, $[Cl^-]_0$ was replaced by a relatively impermeant anion, i.e., SO_4^{2-}, while $[K^+]_0$ and $[Na^+]_0$ were kept constant at 5.8 mM and 140.6 mM respectively. It appears that partial substitution of $[Cl^-]_0$ caused only a small transient depolarization which was returned to the control value by 15—20 min after exposure (Fig. 3). Thus, after 15—20 min in either 63 mM $[Cl^-]_0$ or Cl-free medium, membrane potentials were -25.4 ± 0.3 mV and -27.4 ± 0.4 mV respectively. These potentials were not significantly different ($P > 0.5$) from the control. The resting potential was, therefore, independent of external Cl^- concentration.

Effects of Ca-free solutions

Since it is extensively accepted that the permeability of most biological membranes to ions is determined by $[Ca^{2+}]_0$, it is noteworthy to test whether this is the case in the rabbit lacrimal gland. Table III shows that during exposure to a Ca-free solution the membrane potential was significantly

TABLE III

EFFECT OF Ca-FREE AND Ca-FREE + 5 mM EGTA SOLUTIONS ON THE RESTING MEMBRANE POTENTIAL OF RABBIT LACRIMAL GLAND

Solutions	Membrane potential mean ± S.E. (mV)	Number of observations (n)	Level of significance for difference from the control *
Control	−25.9 ± 0.3	141	
Ca-free	−20.5 ± 0.4	84	$P < 0.001$
Ca-free + 5 mM EGTA	−14.1 ± 0.4	87	$P < 0.001$, $P < 0.001$ **

* P values are from student's t test for unpaired samples.
** Comparison between Ca-free and Ca-free + 5 mM EGTA solutions.

decreased ($P < 0.001$) from the control value of −25.9 ± 0.3 mV to −20.5 ± 0.4 mV. The depolarization of membrane potentials reached steady value within 20 min after the introduction of Ca-free solution. A slight depolarizing effect of Ca-free solution may be, in part, due to the incomplete removal of Ca^{2+} from the immediate external environment of the gland cells or membrane-bound Ca^{2+}. Addition of 5 mM EGTA, indeed, produced more pronounced depolarization. The resting potential was decreased to −14.1 ± 0.4 mV (Table III).

Discussion

The purpose of this study is to determine the ionic dependence of the resting membrane potential of the secretory cells of the lacrimal gland. Although there is no direct evidence as for the site of impalement from the present investigation, we believe that the membrane potential belongs to the acinar and/or intercalary cells for the following reasons: (1) The rabbit lacrimal gland is a tubo-alveolar gland with acini, intercalated ducts, intralobular ducts, and interlobular (excretory) ducts. There are no granulated tubules or striated ducts equivalent to those which are generally found in salivary glands [15,16]. The acinar cells are largest in diameter and by far the most numerous [1,17]. (2) The intralobular and interlobular duct cells are localized deep in the gland tissue as compared to the acinar cells which lie immediately below the surface of the gland. Although our preliminary experiments revealed no correlation between membrane potentials and depth of impalements up to 400 μm from the surface of the gland, we tried not to penetrate the microelectrode deeper than 300 μm, the depth at which the striated duct cells are localized in salivary glands [18—20]. Histological examination of the rabbit lacrimal gland showed that intralobular and interlobular ducts were always localized deep in the gland tissue, more than 300 μm from the free surface (unpublished observations). Most (if not all) impalements were, therefore, in acinar cells and/or intercalary cells. (3) The potential profile, which is unimodal, indicates that the cells being penetrated are homogenous. It is less likely that the duct cells are included in this population since the duct cells should have high membrane potentials such as those in salivary glands [16]. Thus, resting membrane potentials in this study were obtained from the acinar cells and/or the intercalary cells, which are both the secretory unit of the lacrimal gland.

The in vitro resting potential in the present investigation was slightly lower than those reported in vivo [1,2]. Our preliminary experiments indicated that the slight depolarization of membrane potentials in vitro could primarily be explained by the effect of lowering in temperature. When compared to the in vitro studies in other non-excitable cells, the resting potential of rabbit lacrimal gland was lower than those of mouse [21] and rat pancreas [8,22], mouse parotid [23], rabbit gall-bladder [24], rat duodenum [9], rat brown adipose tissue [7], and guinea-pig liver [25]. It was, however, about the same as those of guinea-pig stria cells [26], rat seminiferous tubules [27], and rat epididymal adipose tissue [28]. On the basis of ionic hypothesis of electrical activity of excitable [29] and non-excitable cells [30] resting membrane potentials depend largely on the concentration gradient of K^+ across cell membrane. The resting potential of rabbit lacrimal gland also depends on $[K^+]_0$. A linear relationship between membrane potential and log $[K^+]_0$ was obtained over the range of 5—100 mM $[K^+]_0$ with a slope of 15 mV per 10-fold change in $[K^+]_0$ (Fig. 4). This is very close to the reported value of 15 mV in dog submaxillary gland [31] and 16 mV in rat seminiferous tubular cells [27]. The low value of the slope of potential vs. log $[K^+]_0$ in the lacrimal gland is not surprising since it is typical for the non-excitable cells and may be explained in terms of high Na^+ to K^+ permeability ratio (P_{Na}/P_K) in these cells [30]. In addition, it is likely that the depolarizing effect of $[K^+]_0$ on membrane potentials is complicated by changing $[Na^+]_0$ as evident from the findings that lowering $[Na^+]_0$ produced hyperpolarization of several millivolts (Fig. 5). In fact, the slope was increased to approx. 29 mV in Na-free (Tris replacement) solutions (Fig. 4).

The high P_{Na}/P_K value of rabbit lacrimal gland is also suggested by the fact that membrane potentials are markedly dependent on $[Na^+]_0$. Reduction of $[Na^+]_0$ by replacement with either Tris or choline caused significant hyperpolarization except for Na-free choline solution (Fig. 5). It appears that Tris-for-Na substitution is preferable to choline for Na substitution since the former cause more hyperpolarization than the latter in every instance. The high dependence of the resting potential of lacrimal gland on $[Na^+]_0$ is similar to that of liver cells [32], brown adipose tissue [7], rat seminiferous tubule [27], and rat duodenum [9].

Replacement of external chloride by sulfate appears to cause only transient depolarization. The resting potential returned to the control value by 20 min during prolonged exposure to low or free chloride medium (Fig. 3). The membrane potential of rabbit lacrimal gland is, therefore, independent of $[Cl^-]_0$. The transient depolarization could be explained by outflux of Cl^- into the medium and after redistribution of Cl^- reaches new equilibrium the resting potential is recovered. Thus, Cl^- seems to distribute passively across the membrane of lacrimal gland cells. This kind of interpretation had been made by Hodgkin and Horowicz in frog skeletal muscle [33], by Lamb and Mackinnon in cultured L-cells [34], and by Okada et al. in rat duodenum [9]. Another line of evidence in support to this conclusion is that the equilibrium potential of Cl^- (E_{Cl}) in the rabbit lacrimal gland as calculated from the Nernst equation is —26.4 mV (assuming intracellular Cl^- concentration, $[Cl^-]_i$, 44 mM, from our unpublished observations). It is evident that the observed resting membrane potential in vitro (—26.7 mV) is almost identical to the calculated E_{Cl}.

Since the membrane potential of rabbit lacrimal gland depends primarily on $[K^+]_o$ and $[Na^+]_o$ whereas $[Cl^-]_o$ appears to have virtually no effect, the Goldman equation for the effective emf (E) of lacrimal cell membrane may be applied and can be used in the following form:

$$E = 59 \log \frac{[K^+]_o + \alpha[Na^+]_o}{[K^+]_i + \alpha[Na^+]_i} \quad (1)$$

at 25° C where α is the ratio of the permeability coefficient (P_{Na}/P_K). The value for $[K^+]_i$ and $[Na^+]_i$ in the rabbit lacrimal gland from our unpublished observations were approx. 150 mM and 23 mM respectively. Assuming the intracellular K^+ and Na^+ of the lacrimal gland being evenly distributed throughout the cell, the ratio of the permeability coefficient (α) was found from Eqn. 1 to be 0.35 for the cell incubated in normal Krebs-Ringer bicarbonate solution. This value is about 35 times greater than that of skeletal muscle but it is commensurate with the low resting potential in this gland and compatible with those of the non-excitable cells with comparable membrane potentials [16,30,32].

Omission of calcium from superfusion solution produced small but significant depolarization of the resting potential (Table III) similar to those reported in several other tissues [10,21,27,35]. According to Brink [36] and Shanes [37] the depolarizing effect of calcium could be explained by a gain of $[Na^+]_i$ and/or a loss of $[K^+]_i$ resulting from an increase in cell membrane permeability as membrane-bound calcium is removed. It is unlikely that changes in $[Ca^{2+}]_o$ could directly alter membrane potentials since the ionic gradient of calcium across cell membrane (ratio of $[Ca^{2+}]_o$ to $[Ca^{2+}]_i$) is 10^2—10^5, which is in the same direction but much greater in magnitude than that of sodium [38]. If the membrane is also permeable to calcium, reduction of $[Ca^{2+}]_o$ would cause hyperpolarization (not depolarization).

In conclusion, we have shown that, in the rabbit lacrimal gland in vitro, the acinar and/or intercalary cells have low resting membrane potentials which are primarily dependent on the external potassium, sodium, and calcium ions but are independent of the external chloride ion. The data suggest that the resting cell membrane of rabbit lacrimal gland is highly permeable to both potassium and sodium, and the P_{Na}/P_K was estimated to be 0.35. Since variation of the external chloride concentration produced only a transient change in membrane potentials it is concluded that chloride passively distributes across the cell membrane of rabbit lacrimal gland.

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