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MEMBRANE POTENTIAL, RESISTANCE, AND INTERCELLULAR COMMUNICATION IN THE LACRIMAL GLAND: EFFECTS OF ACETYLCHOLINE AND ADRENALINE

BY N. IWATSUKI* AND O. H. PETERSEN
From the Department of Physiology, the University, Dundee DD1 4HN

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SUMMARY

1. Intracellular micro-electrode recordings were made from surface acini of mouse exorbital lacrimal glands placed in a Perspex bath through which oxygenated physiological saline solutions were circulated. Two micro-electrodes were inserted into neighbouring communicating cells. Through one of the electrodes, current pulses could be injected. The cells impaled were stimulated by iontophoresis of acetylcholine (ACh), adrenaline or isoprenaline from an extracellular micro-pipette.

2. During exposure to standard Krebs solution the resting membrane potential was $-42{\cdot}5$ mV $\pm$ 1·2 and the resting input resistance 3·3 MΩ $\pm$ 0·3. When the tips of the two intracellular micro-electrodes were more than 100 μm apart no electrical coupling between two impaled cells could be detected. At intertip distances below about 80 μm coupling was frequently observed. In all such cases the coupling ratio was 1. The resting current–voltage relation was almost linear within the membrane potential range of -30 to -80 mV.

3. During exposure to standard Krebs solution short iontophoretic pulses of ACh or adrenaline caused fully reversible hyperpolarizations accompanied by marked reduction of surface cell membrane resistance and membrane time constant. The effects of ACh were blocked by atropine ($1{\cdot}4 \times 10^{-6}$ M). Iontophoresis of isoprenaline never had any detectable effect on membrane potential or resistance.

4. Applying de- or hyperpolarizing direct currents through one of the two intracellular micro-electrodes the effect of ACh or adrenaline could be observed at different levels of resting potential. Depolarizing the acinar cell membrane resulted in an enhanced stimulant-evoked hyperpolarization whereas hyperpolarizing the acinar cell membrane resulted in a reduction, and at potentials more negative than -60 mV in a reversal of the stimulant-evoked potential change. The ACh equilibrium potential (E_{ACh}) under control conditions was $-56{\cdot}6$ mV $\pm$ 1·1 and $E_{Adrenaline}$ was $-61{\cdot}4$ mV $\pm$ 1·0.

5. Replacing the control superfusion solution by a Cl-free sulphate solution resulted in an immediate shift of E_{ACh} towards more negative values. At steady state in the Cl-free solution the resting input resistance was 6·8 MΩ $\pm$ 1·3. E_{ACh} was $-95{\cdot}9$ mV $\pm$ 3·4.

6. Reducing $[K]_o$ from the usual 4·7 to 1·0 mM resulted in an immediate marked

* M.R.C. Post-doctoral Research Fellow.

increase in the amplitude of ACh-evoked hyperpolarization whereas increasing $[K]_o$ to 10 mM almost abolished the ACh-evoked potential, but not resistance change.

7. It is concluded that both ACh and adrenaline act on the lacrimal acinar cell membrane by opening up pathways mainly permeable to K, but also somewhat permeable to Na. This results in a release of K and an uptake of Na into the acinar cells.

INTRODUCTION

The lacrimal gland produces an isotonic fluid with a plasma-like Na concentration (Thaysen & Thorn, 1954; Thaysen, 1960; Alexander, van Lennep & Young, 1972) and secretes protein (peroxidase) by the process of exocytosis (Hertog, Sies & Miller, 1976; Keryer & Rossignol, 1976). Both secretory processes appear to be controlled by an action of actylcholine (ACh) on the lacrimal acinar cell membrane (Thaysen, 1960; Hertog *et al.* 1976; Keryer & Rossignol, 1976).

Parasympathetic nerve stimulation *in vivo* or ACh addition *in vitro* causes membrane potential changes in lacrimal acinar cells. In the cat, these are complex consisting mostly of an initial depolarization followed by hyperpolarization (Hisada & Botelho, 1968) whereas in the rabbit ACh simply evokes hyperpolarization (Kikkawa, 1970; Dartt & Botelho, 1977). Results on measurements of membrane resistance and resistance changes following stimulation have not previously been reported.

In order to obtain more precise information on the ionic currents underlying the stimulation-evoked membrane potential changes in lacrimal acinar cells the present study on the mouse exorbital lacrimal gland was undertaken. We have used a technique with two separate intracellular micro-electrodes placed in neighbouring electrically coupled surface cells, an approach recently developed by us in a study of ACh effects on pancreatic acinar cells (Iwatsuki & Petersen, 1977*b*).

METHODS

Isolated exorbital lacrimal glands from mice were mounted in a Perspex bath superfused with physiological saline solutions at 37 °C as previously described for the pancreas (Nishiyama & Petersen, 1974*a*). The standard Krebs bicarbonate solution was the one usually employed in this laboratory (Nishiyama & Petersen, 1974*a*; Iwatsuki & Petersen, 1977*a*). The various ionic replacements have also been described previously (Nishiyama & Petersen, 1975; Iwatsuki & Petersen, 1977*b*).

The micro-electrode set-up and recording arrangement was identical to that used by Iwatsuki & Petersen (1977*b*). Under visual control (Leitz, stereomicroscope, 160 ×) two micro-electrodes (filled, using the fibre glass method, with 3 M-KCl or 5 M-K acetate, and bevelled (Iwatsuki & Petersen, 1978)) were inserted into surface acinar cells. After insertion the tips of the two micro-electrodes were usually 30–80 μm apart. Stimulation was carried out by micro-iontophoresis from an extracellular micro-pipette filled with either 2 M-AChCl, 1 M-adrenaline bitartrate or 1 M-isoprenaline hydrochloride as already described (Iwatsuki & Petersen, 1977*a*).

RESULTS

The resting properties

The mean resting membrane potential was $-42{\cdot}5$ mV $\pm$ 1·2 (S.E.) (n = 30) and the mean input resistance 3·3 MΩ $\pm$ 0·3 (n = 30).

In all cases in which electrical coupling between neighbouring cells was observed the coupling ratio (current pulse-induced potential change in cell of current injection divided by electrotonic potential change in neighbouring cell) was 1 or very close to 1 (Figs. 2, 4, 5). Coupling was only observed when the distance between the tips of the two micro-electrodes impaled was less than 100 μm.

Fig. 1 shows the resting current–voltage relation obtained by injecting rectangular de- or hyperpolarizing current pulses through one micro-electrode and recording the potential change with the other electrode. The relationship was slightly

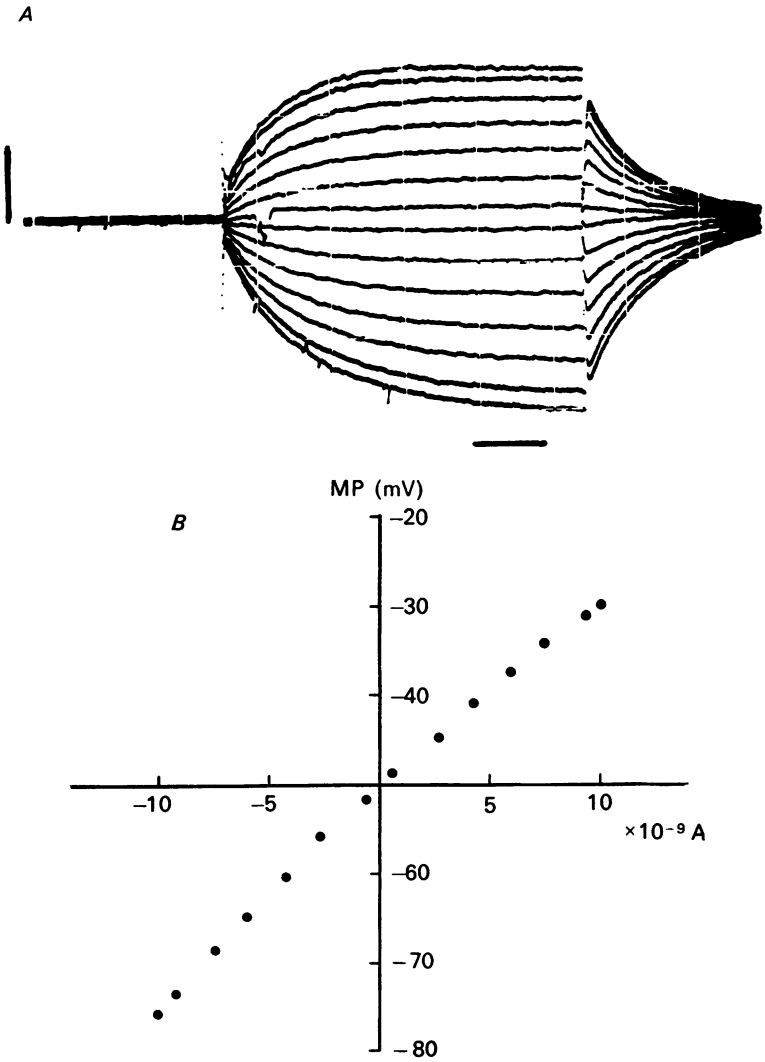

Fig. 1. The resting current–voltage relationship in lacrimal acinar cells. *A*, membrane potential (MP) changes in response to rectangular 100 msec de- or hyperpolarizing current pulses (not shown) of different magnitude. Calibration: horizontal 20 msec, vertical 10 mV. Two intracellular electrodes were used, one for current passage and the other for recording. *B*, plot of membrane potential (steady state) as a function of magnitude and polarity of injected current. The points have been obtained from the experiment shown in *A*.

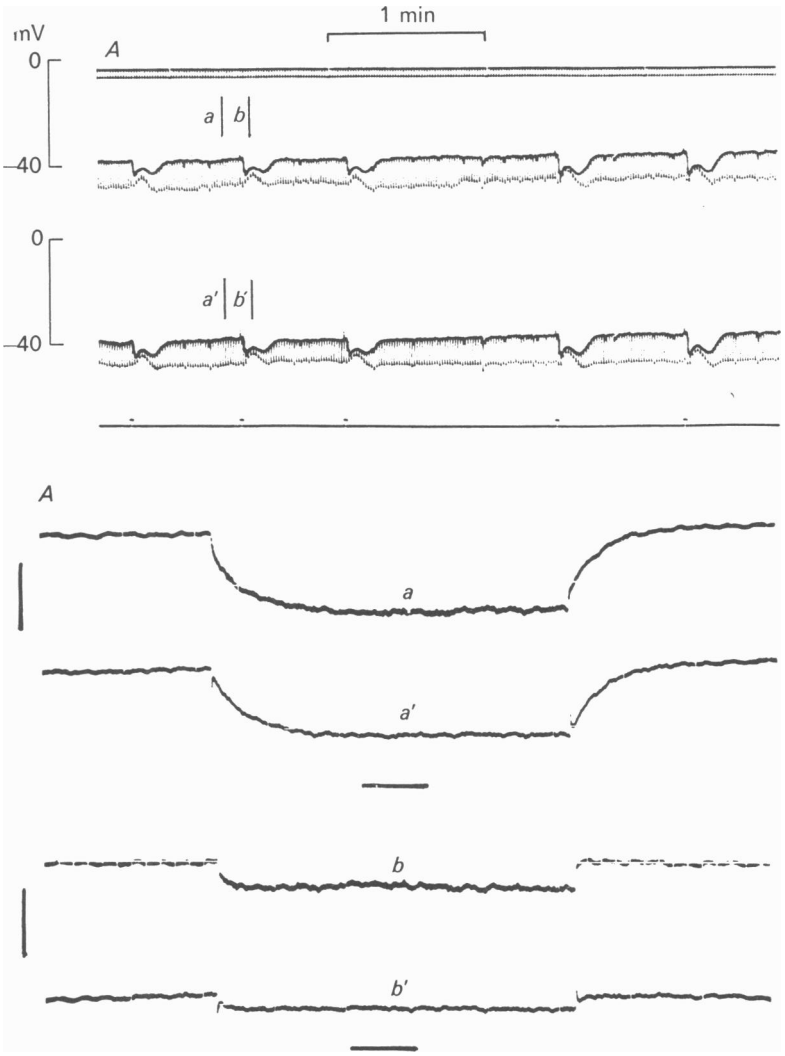

Fig. 2. The effect of microiontophoretic application of ACh on membrane potential and resistance in two communicating cells. Rectangular current pulses (100 msec, $2{\cdot}5 \times 10^{-9}$ A) were repetitively injected through the micro-electrode recording the membrane potential represented by the upper potential traces. Ejecting ACh current pulses (80 nA, 20 nA retaining current) were passed through the extracellular AChCl-filled micro-pipette at signals in bottom traces. The duration of ejecting currents was 1 sec except in those cases in which another figure (sec) is written under the signal. The oscilloscope photographs show the time course of current-pulse induced membrane hyperpolarizations in the two cells at times a/a' and b/b' in A. Calibration: horizontal 20 msec, vertical 10 mV. A, B, C and D are consecutive records from the same pair of cells. Interval between A and B about 5 min. Interval between B and C about 2 min. Interval between C and D 15 min. In D the membrane potential was severely reduced and shortly afterwards the units were lost. At that point the two cells had been impaled for about 45 min. Atropine had clearly not been entirely washed out of the preparation in D since very long pulses of ACh application were needed to obtain an effect.

assymetrical since the resistance was higher for the hyperpolarizing than for the depolarizing currents. This was the case in all the three records of this type obtained.

In some cells spontaneous short-lasting hyperpolarizations were observed. These potential changes were blocked by atropine ($1{\cdot}4 \times 10^{-6}$ M) (Fig. 2).

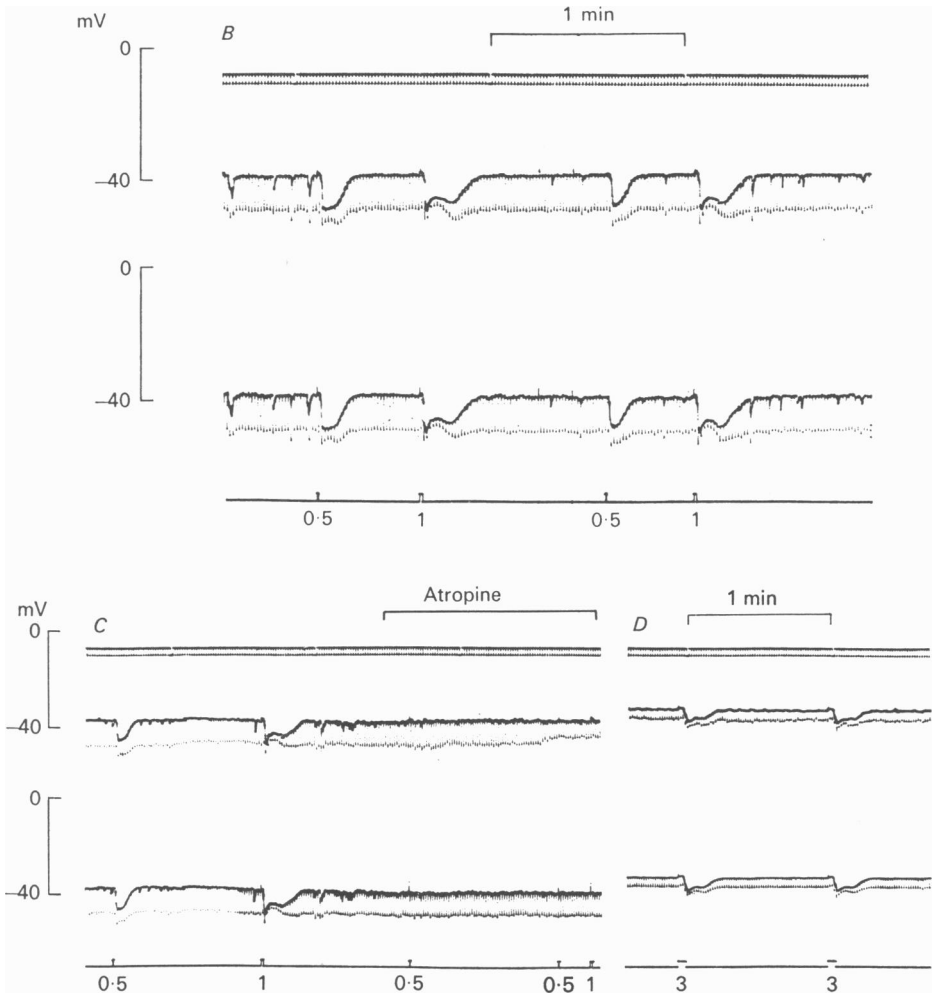

Fig. 2*B*, *C* and *D*. For legend see facing page.

The effect of ACh

Iontophoretic pulses of ACh caused hyperpolarization accompanied by a marked reduction in surface membrane resistance and a shortening of the electrical time constant of the membrane (Fig. 2). Short pulses (0·5 sec) caused a simple monophasic hyperpolarization. In this type of response the maximum intracellular negativity was reached at the same time as the minimum input resistance. Somewhat longer pulses (1 sec) resulted in the same initial response but were followed by a further increase in conductance reaching its peak after the potential had attained a level less negative than at the peak of the first phase. This ACh-evoked membrane

conductance increase occurring in two stages was finally followed by a second hyperpolarization reaching its peak at a time when the resistance was returning to the pre-stimulation level (Fig. 2). ACh effects of the type shown in Fig. 2 were recorded from seventeen cells in six glands. The mean value of the initial ACh-evoked hyperpolarization following maximal stimulation (80 nA, 1 sec (20 nA retaining current)) was 9·1 mV ± 0·8 accompanied by a resistance reduction from 3·4 MΩ ± 0·4 to 0·5 MΩ ± 0·1 (n = 17). The effects of ACh were blocked reversibly by atropine (Fig. 2).

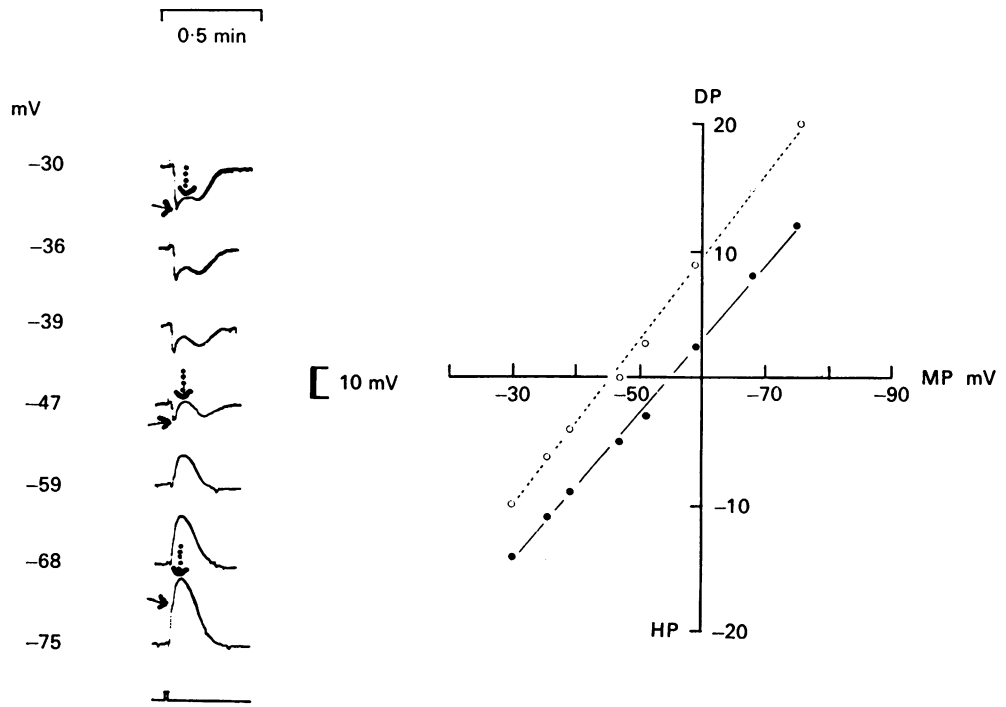

Fig. 3. The measurement of the ACh equilibrium potential. Direct current (de- or hyperpolarizing) was passed through one intracellular electrode while the potential was recorded through the other. To the left is shown the effect of ACh (80 nA ejecting, 1 sec; retaining current 20 nA) at different levels of resting potentials (figures written to the left of records). The potential change at end of phase 1 (fully drawn arrows) and end of phase 2 (dashed arrows) plotted as a function of resting potential is shown to the right. Filled circles represent phase 1 and open circles phase 2. DP means depolarizing, HP hyperpolarizing and MP resting membrane potential.

The ACh equilibrium potential

Passing direct current (de- or hyperpolarizing) through one intracellular micro-electrode, and recording with another intracellular micro-electrode from a neighbouring coupled cell, made it possible to set the resting potential at different levels and observe the effect of ACh over a wide range of resting potentials. Fig. 3 shows an example of this. At the spontaneous resting potential of −39 mV ACh evoked hyperpolarization. Increasing intracellular negativity reduced the amplitude of the initial ACh-evoked hyperpolarization and finally at high resting potentials reversed the potential change into a depolarization. Reducing the intracellular negativity

by passing depolarizing current enhanced the amplitude of the ACh-evoked hyperpolarization. As already mentioned above it was possible to distinguish between two stages in the ACh-evoked conductance change and the equilibrium potential for both phases was determined. The two stages are easily recognized at $E_m = -47$ mV (Fig. 3). At this resting potential there is an initial hyperpolarization of 5 mV whereas at the peak of stage 2 the potential has returned to the pre-stimulation level. At the higher resting potentials it is more difficult to distinguish between phase 1 and 2 but even here a small inflexion (marked by arrow in Fig. 3) can be seen at some point during the ACh-evoked depolarization. In Fig. 3 is plotted the ACh-evoked potential change in phase 1 and 2 as a function of the resting potential. The difference between E_{ACh} (the intercept with the x-axis) at the maximum of phase 1 and 2 was not very marked, but in all ten cells investigated E_{ACh} in phase 2 was less negative than in phase 1, i.e. E_{ACh} changed with time. The mean value of E_{ACh} (phase 1) was $-56{\cdot}6$ mV $\pm$ 1·1 ($n = 10$) and in phase 2 $-51{\cdot}0$ mV $\pm$ 1·3 ($n = 10$).

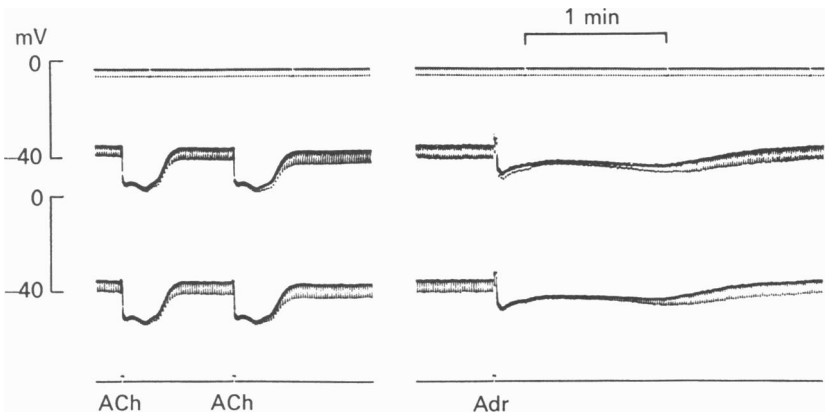

Fig. 4. The effect of ACh (80 nA, 1 sec; retaining current 20 nA) and adrenaline (500 nA, 1 sec; retaining current 10 nA) in the same two communicating cells. Interruption of traces represents an interval of 1·5 min. Rectangular current pulses (100 msec, 2×10^{-9} A) were repetitively injected through electrode recording membrane potential represented by upper potential trace.

The effect of adrenaline

The effect of adrenaline was essentially similar to that of ACh causing an initial hyperpolarization accompanied by marked resistance reduction followed by a secondary hyperpolarization reaching its peak at a time when the resistance was returning towards the pre-stimulation level (Fig. 4). In some cases we were able to observe the effect of ACh and adrenaline in the same pair of cells (Fig. 4). The effects are clearly very similar the adrenaline effect only being slightly more prolonged. The mean value of the initial adrenaline-evoked hyperpolarization following maximal stimulation (500 nA, 1 sec (10 nA retaining current)) in thirteen cells was 9·2 mV $\pm$ 2·0 accompanied by a resistance reduction from 3·2 MΩ $\pm$ 0·8 to 0·8 MΩ $\pm$ 0·2. The effect of isoprenaline was tested in five cells; in all cases without any effects being observed. Fig. 5 shows an example of an experiment in which adrenaline evoked the usual hyperpolarization but in which isoprenaline was without

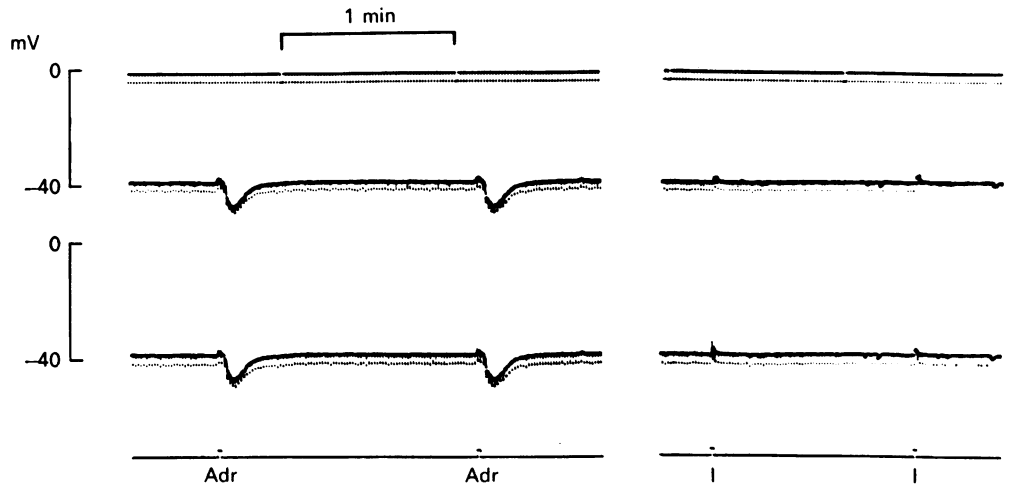

Fig. 5. The effect of adrenaline (Adr) (1000 nA, 1 sec; retaining current 10 nA) and isoprenaline (I) (1000 nA, 1 sec; retaining current 10 nA) in the same two communicating cells. Rectangular current pulses (100 msec, 2×10^{-9} A) were repetitively injected through electrode recording membrane potential represented by upper potential trace. Interval of 2 min at interruption of traces.

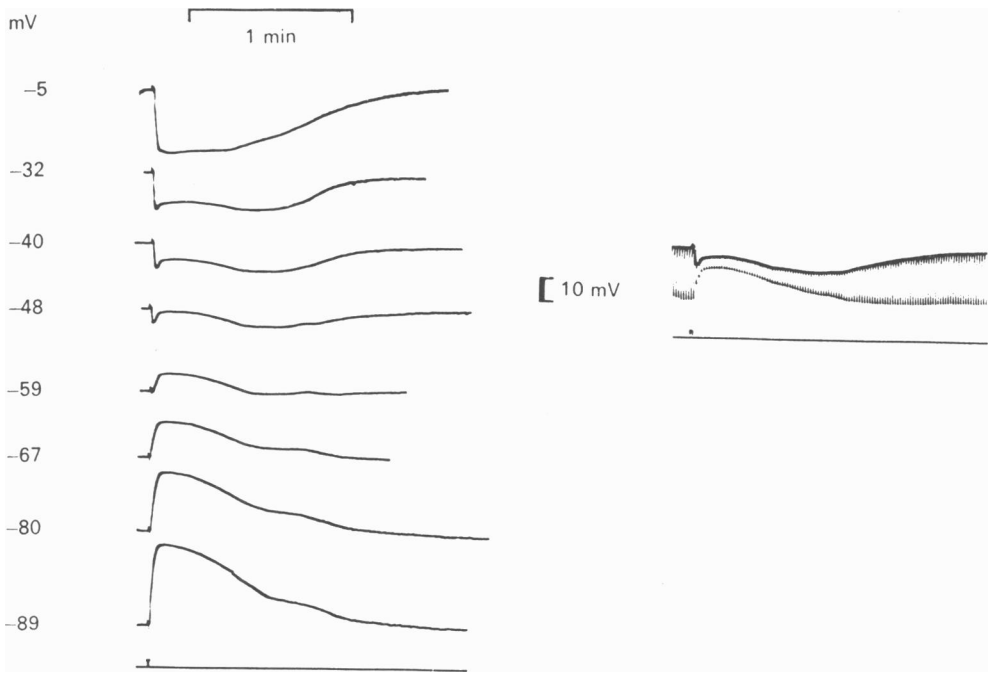

Fig. 6. The effect of adrenaline (500 nA, 0·5 sec; retaining current 10 nA) at different levels of resting potential (figures written to the left of individual records) in the same cell. To the right is shown the effect of the same dose of adrenaline in the same cell during repetitive injections of rectangular current pulses (100 msec, $3{\cdot}8 \times 10^{-9}$ A) into a neighbouring coupled cell. The spontaneous resting potential was -46 mV.

effect. In a parallel series of experiments performed on the mouse parotid with exactly the same technique we had no difficulty in observing depolarizing effects of isoprenaline (Roberts & Petersen, 1978).

The adrenaline equilibrium potential

Fig. 6 shows an example of the measurement of E_{adr} using exactly the same technique as described above for ACh. The mean value of E_{adr} was $-61{\cdot}4$ mV $\pm$ 1·0 for phase 1 and $-52{\cdot}8$ mV $\pm$ 2·4 ($n = 9$) for phase 2. In a number of cases E_{ACh} and E_{adr} were determined in the same cell and in all these cases the values obtained

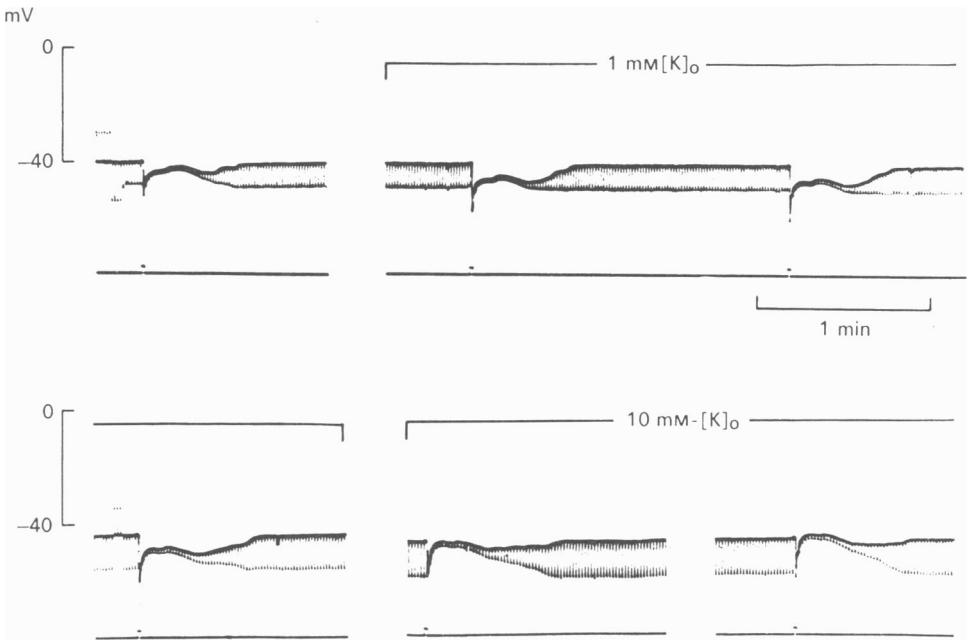

Fig. 7. The effect of varying $[K]_o$ on the ACh-evoked potential and resistance change. Rectangular current pulses (100 msec duration and 2×10^{-9} A in upper two traces and $2{\cdot}5 \times 10^{-9}$ A in lower three traces) were repetitively injected through a second micro-electrode inserted into a neighbouring cell. The interruptions of the tracings represent intervals of about 2 min. At marker signals in bottom traces ACh (80 nA, 1 sec; retaining current 20 nA) was applied.

were almost identical. Fig. 6 also shows an example of the assessment of E_{adr} using short pulses of current repetitively injected through one micro-electrode while recording with another intracellular electrode. In this case it is possible to obtain a range of membrane potentials within which E_{adr} can be found. The change in E_{adr} with time is clearly seen in this record (Fig. 6).

The effect of varying $[K]_o$

Fig. 7 shows an example of the effect of first reducing $[K]_o$ from the normal 4·7 to 1 mM and subsequently increasing $[K]_o$ to 10 mM. The effects of higher K concentrations were not explored because of the possible danger of endogenous neurotransmitter release induced by K depolarization of nerve terminals within

the gland tissue. Reducing $[K]_o$ immediately enhanced the hyperpolarizing effect of ACh. The subsequent increase of $[K]_o$ to 10 mM caused a slight hyperpolarization (possibly due to activation of an electrogenic Na pump) and the hyperpolarizing effect of ACh was almost abolished while the resistance reduction was very clearly seen. Three records almost identical to the one presented in Fig. 7 were obtained.

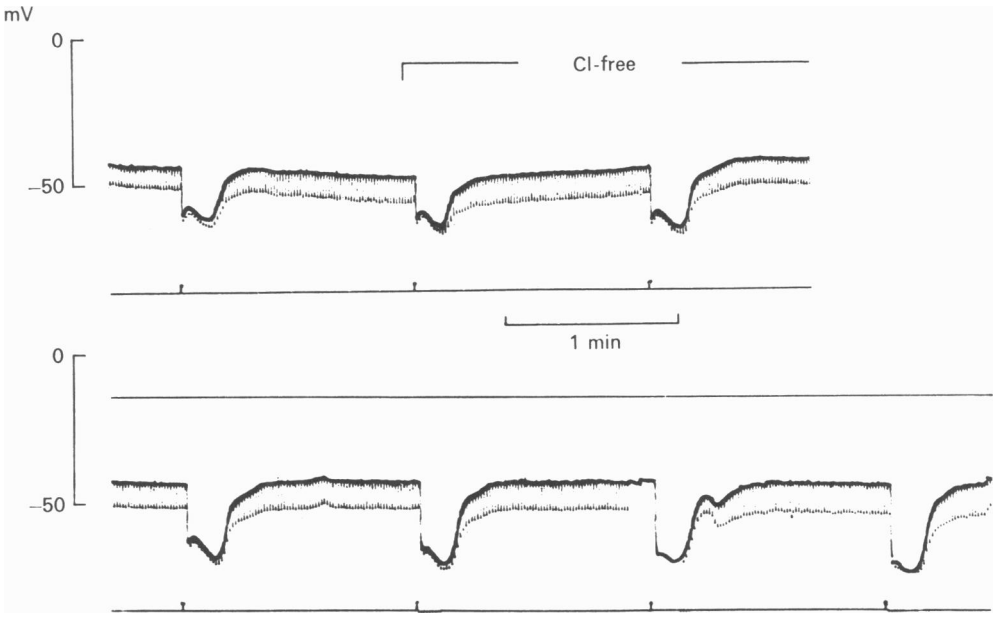

Fig. 8. The effect of omitting superfusion fluid Cl on ACh-evoked potential and resistance change. Rectangular current pulses (100 msec, 2×10^{-9} A) were repetitively injected through another micro-electrode inserted into a neighbouring cell. At marker signals in bottom traces ACh (80 nA, 0·5 sec; retaining current 20 nA) was applied. The lower panel represents an immediate continuation of the upper panel.

The effect of omitting external Cl

Changing the bath superfusion fluid from the standard Cl-containing solution to a Cl-free sulphate solution had little effect on the resting potential apart from a small transient depolarization. The mean resting potential after half an hour of exposure to the Cl-free sulphate solution was $-45{\cdot}1$ mV $\pm$ 2·0 and the mean resistance 6·8 MΩ $\pm$ 1·3. Immediately after taking away the superfusion fluid Cl the initial ACh-evoked hyperpolarization increased in amplitude, thereafter there was a progressive increase in ACh-evoked hyperpolarization with time (Fig. 8) until after about 30 min a new steady state had been reached. The mean value of maximal ACh-evoked initial hyperpolarization was 29·6 mV $\pm$ 3·3. This hyperpolarization was accompanied by a reduction in resistance from 6·8 $\pm$ 1·3 to 1·7 MΩ $\pm$ 0·6 (n = 9). E_{ACh} was measured in five cells after 30–60 min of exposure to Cl-free sulphate solution. Fig. 9 shows an example of a measurement of E_{ACh} and a plot of the data obtained from the same experiment. The mean value of E_{ACh} measured in the Cl-free solution was $-95{\cdot}9$ mV $\pm$ 3·4 (phase 1) and $-91{\cdot}7$ mV $\pm$ 3·1 (phase 2).

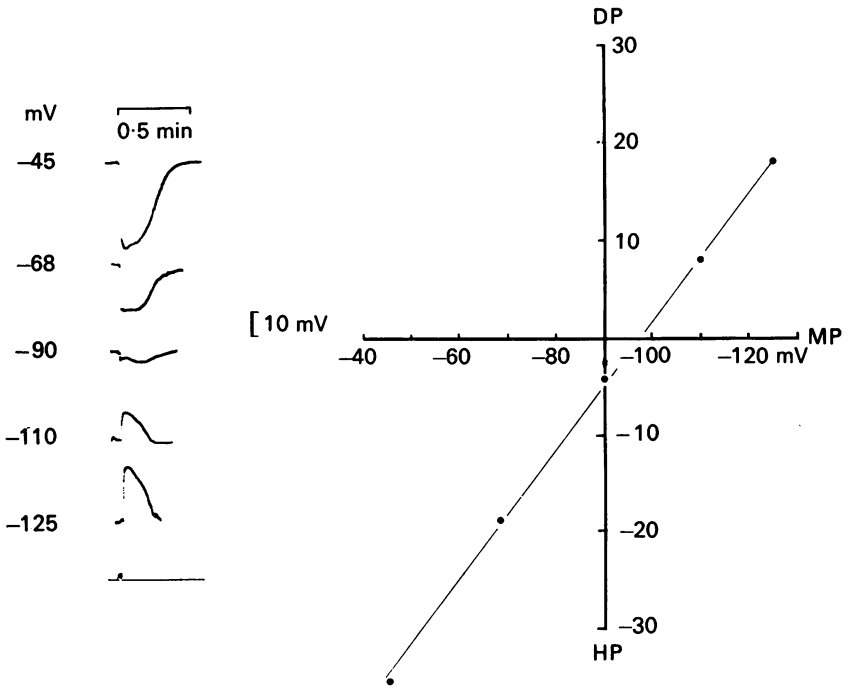

Fig. 9. The acetylcholine equilibrium potential during exposure of the tissue to Cl-free solution. To the left is shown the effect of ACh (80 nA, 1 sec; retaining current 20 nA) at different levels of resting potential (figures written to the left of the individual records). The initial potential change as a function of resting potential, is shown to the right. DP means depolarization, HP hyperpolarization and MP resting membrane potential.

DISCUSSION

The mean resting membrane potential of lacrimal acinar cells in the cat *in vivo* and the rabbit *in vitro* has been reported as -28 and -25 mV, respectively (Hisada & Botelho, 1968; Pholpramool & Korppaibool, 1977) whereas in the rabbit lacrimal gland *in vivo* a mean resting potential of -37 mV was obtained (Kikkawa, 1970). In this study of mouse lacrimal acinar cells *in vitro* the mean resting potential was -43 mV, which is a value very similar to that of mouse and rat pancreatic acinar cells *in vitro* or *in vivo* (Matthews & Petersen, 1973; Petersen & Ueda, 1975), but considerably lower than the -69 mV found in the mouse parotid (Pedersen & Petersen, 1973). The stability of membrane potential recording, even with simultaneous registration from two neighbouring cells, was very much better in this study than in those previously published.

Neighbouring lacrimal acinar cells within a restricted area, probably corresponding to an acinus or a small group of neighbouring acini are fully electrically coupled. Over long distances ($> 100\ \mu$m), however, there is no coupling at all. These findings are similar to those made previously on cockroach salivary glands (Ginsborg, House & Silinsky, 1974) and the mammalian pancreas (Petersen & Ueda, 1976; Iwatsuki & Petersen, 1977*b*). In contrast it is possible to observe electrical coupling over long distances (> 4–$500\ \mu$m) in mouse liver (Petersen, 1976).

Changes in lacrimal acinar cell membrane potential following stimulation of the lacrimal nerve *in vivo* or intra-arterial injection of ACh or adrenaline *in vivo* or in the perfused gland have been reported (Hisada & Botelho, 1968; Kikkawa, 1970; Dartt & Botelho, 1977). In the cat there seemed to be a transient depolarization followed by hyperpolarization (Hisada & Botelho, 1968) whereas in the rabbit the response to ACh was simply hyperpolarization. Adrenaline in most cases caused depolarization (Kikkawa, 1970). Our results indicate that in the mouse ACh and adrenaline have the same action, namely hyperpolarization accompanied by marked resistance reduction. With short iontophoretic pulses monophasic hyperpolarizations are seen, but with larger pulses a secondary hyperpolarization occurs at a time when the resistance is returning to pre-stimulation levels. The mechanism underlying this delayed hyperpolarization has not been analysed, but since it resembles closely what can be observed in the parotid (Roberts & Petersen, 1978) where an electrogenic sodium pump is the cause, it seems likely that it could be due to active electrogenic Na extrusion. Electrogenic Na pumps are well established in several mammalian gland cell types (Petersen, 1976).

The initial stimulation-evoked hyperpolarization occurring in two phases (Figs. 2, 3) has been investigated in some detail. E_{ACh} as well as E_{adr} is about -60 mV during control conditions. Intracellular concentrations, unfortunately only available for the rabbit lacrimal gland (Pholpramool & Korppaibool, 1977), are very similar to figures obtained from mouse and rat pancreas and rat salivary glands: $[Na]_i = 23$ mM, $[Cl]_i = 44$ mM and $[K]_i = 150$ mM. At the equilibrium potential there is zero net flux through ACh-opened pathways and using concentrations rather than activities the following equation (Goldman, 1943; Hodgkin & Katz, 1949) can be derived from the Nernst–Planck electrodiffusion equation assuming a constant electrical field through the membrane and only taking account of Na, K and Cl:

$$E_{ACh} = -57 \text{ mV} = 61{\cdot}5 \log \frac{P_{A_K} 4{\cdot}7 + P_{A_{Na}} 146 + P_{A_{Cl}} 44}{P_{A_K} 150 + P_{A_{Na}} 23 + P_{A_{Cl}} 115} \text{ mV}, \quad (1)$$

where P_A stands for permeability of ACh-opened pathways.

In the absence of Cl the equation reads:

$$E_{ACh} = -96 \text{ mV} = 61{\cdot}5 \log \frac{P_{A_K} 4{\cdot}7 + P_{A_{Na}} 182}{P_{A_K} 150 + P_{A_{Na}} 23} \text{ mV}. \quad (2)$$

From these two equations we can calculate the apparent $P_{A_{Cl}}/P_{A_K}/P_{A_{Na}}$ (Iwatsuki & Petersen, 1977*b*) if we assume that exposure to Cl-free solution has no effect on $P_{A_{Na}}$ or P_{A_K} and on $[K]_i$ and $[Na]_i$. The result obtained is $P_{A_{Cl}}/P_{A_K}/P_{A_{Na}} = 1/2/0$. This then indicates a marked Cl permeability for the ACh-opened pathways.

This result is, however, not in agreement with the transients obtained by changing from control to Cl-free solution (Fig. 8). Immediately after removing superfusion fluid Cl there must be a very large gradient favouring Cl efflux from the acinar cells. If ACh-opened pathways were markedly permeable to Cl, E_{ACh} should move in the positive direction in this period. This is clearly not the case. Indeed the ACh-evoked hyperpolarization immediately increased in size following extracellular Cl removal. This seems to indicate that by removing extracellular Cl the ACh-evoked increase in P_{A_K} becomes dominant. This could be explained by assuming that the low Cl solution increased $P_{A_K}/P_{A_{Na}}$. On the basis of indirect evidence

this explanation was given in connexion with a discussion of ACh effects on mouse submaxillary gland. The resistance of the ACh-opened pathways was 4 times higher during exposure to Cl-free than during exposure to control solution. $P_{A_{Na}}$ must therefore have been reduced rather than P_{A_K} enhanced by the removal of Cl. Since there seems to be some evidence for a very low $P_{A_{Cl}}$ we have repeated the calculations of $P_{A_{Na}}$ and P_{A_K} using (1) and (2) assuming this time $P_{A_{Cl}} = 0$. In this case $P_{A_{Na}}/P_{A_K} = 0{\cdot}1$ during exposure to control solution. During exposure to Cl-free solution ACh only increases K permeability, i.e. $P_{A_{Na}} = 0$. This result may reflect the real situation better than the first result given above.

The potential and resistance changes in lacrimal acinar cells are remarkably similar to those observed in salivary glands (Nishiyama & Petersen, 1974*b*; Roberts & Petersen, 1978). The main difference between the lacrimal and the parotid glands is the somewhat lower resting potential in the lacrimal gland, and the lack of an isoprenaline effect. In the salivary glands it is well known that ACh and α-adrenergic agents cause K release accompanied by Na uptake (Petersen, 1970, 1971; Poulsen, 1974). The present electrophysiological data would indicate that such a passive Na/K exchange also occurs in the lacrimal gland following stimulation. The marked effect of varying $[K]_o$ on the ACh-evoked potential change (Fig. 7) supports the conclusion that ACh opens up pathways mainly permeable to K. Since E_{ACh} is considerably less negative than E_K and it appears that $P_{A_{Cl}}$ is low the ACh-opened pathways must, however, have a considerable Na permeability.

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