Activation of Ca^{2+} -dependent Cl^- and K^+ Conductances in Rat and Mouse Parotid Acinar Cells

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Abstract Isolated acinar cells from rat and mouse parotid glands were studied with patch-clamp whole-cell current recordings. Acetylcholine (ACh) stimulation caused a transient inward current at a membrane potential of -70 mV, and a sustained outward current at a membrane potential of 0 mV, in quasi physiological Na^+ , K^+ ion gradients, except the zero- Cl^- ion gradient condition across the membrane. The reversal potential obtained from the ACh-evoked steady current was about -75 mV, in this ionic condition. When major Cl^- ions of both the pipette and the bath solution were replaced, either by glutamate or by sulphate, only a large outward current was observed, at a membrane potential of -60 mV, in the presence of ACh. The addition of Ca^{2+} -ionophore A23187 caused responses similar to those evoked by ACh. The reversal potential of A23187-induced current was close to the K^+ equilibrium potential of -90 mV, in a Cl^- -free condition. When K^+ -free NaCl solution was used in the pipette and the bath, A23187 caused only a large inward current, at a membrane potential of -60 mV. The reversal potential of A23187-evoked current was about -15 mV, in a symmetrical K^+ -free, NaCl condition. These results suggest that the ACh and A23187 activate Cl^- as well as K^+ conducting pathways via an increase in $[\text{Ca}^{2+}]_i$ in the parotid acinar cells. The A23187-evoked large K^+ current could not be explained solely by a rise in open probability of the channels.

Key words: parotid acinar cell, patch clamp, K^+ channel, Cl^- channel, Ca^{2+} activation.

The agonist-evoked primary electrolyte secretion from parotid gland acinar cells has a plasma-like Na^+ and Cl^- composition (SCHNEYER *et al.*, 1972; YOUNG and VAN LENNEP, 1979). Conventional microelectrode studies of mouse parotid gland acinar cells have shown that the autonomic nerve stimulation, as well as the application of acetylcholine (ACh) or adrenaline, increases the membrane con-

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ductance primarily to K^+ but also to other ions (ROBERTS *et al.*, 1978; GALLACHER and PETERSEN, 1980). The recent patch-clamp single channel current recordings have shown that Ca^{2+} - and voltage-activated K^+ channels, with a large unit conductance, dominate in the basolateral membrane in these glands (MARUYAMA *et al.*, 1983a). PETERSEN and MARUYAMA (1984) have considered that a Na-K-Cl cotransport system, together with K^+ channels on the basolateral membrane, is relevant to electrolyte secretion in the salivary gland acinar cells. They have proposed that K^+ efflux through these K^+ channels, and K^+ reuptake by the Na-K-Cl cotransporter, are responsible for the rate of NaCl uptake into the acinar cells.

In this report we shall demonstrate that ACh and Ca^{2+} -ionophore A23187 (A23187) increases Cl^- as well as K^+ conductance, through an increase in intracellular ionized calcium ($[Ca^{2+}]_i$), in mouse and rat parotid gland acinar cells. This is an important observation, since the Cl^- channels may be directly associated with Cl^- secretion into the lumen, if these channels are present on the luminal membrane. In recent patch-clamp studies on lacrimal gland acinar cells, a Ca^{2+} -activated Cl^- conducting pathway has been reported (MARTY *et al.*, 1984; FINDLAY and PETERSEN, 1985).

MATERIALS AND METHODS

Single acinar cells were enzymatically isolated from the rat and mouse parotid glands with a serial treatment of collagenase (200 unit/ml, Sankyo) and trypsin (3 mg/ml, Sigma type III-S), dissolved in a physiological saline. The method for isolating the cells was essentially similar to that described by FINDLAY (1984). Patch-clamp whole-cell current and single-channel current recordings were established by the standard patch-clamp techniques (HAMILL *et al.*, 1981). All experiments were carried out at room temperature (20–22°C). Currents were recorded with a L/M EPC-5 (List-Electronic, Darmstadt, F.R.G.), or a S-3666 (Nihonkohden, Japan) amplifier. The glass pipettes, with a resistance of 2 to 4 M Ω , were used to obtain "giga seal" from single parotid acinar cells placed in a 1 ml capacity chamber. The standard bathing solution contained (mM); 140 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.13 $MgCl_2$, 10 glucose, and 10 HEPES-NaOH (pH 7.2). The standard pipette solution contained (mM); 145 KCl, 1.13 $MgCl_2$, 10 glucose, 1 EGTA, and 10 HEPES-KOH (pH 7.2). The calcium concentration in the pipette solution was less than 10^{-9} M (FINDLAY, 1984). The Cl^- -free bathing solution had the following composition (mM); 110 Na_2SO_4 or 140 Na-glutamate, 4.7 KCl, 1.13 $MgCl_2$, 2.5 $CaCl_2$, 10 glucose, and 10 HEPES-NaOH (pH 7.2). The Cl^- -free pipette solution contained (mM); 110 K_2SO_4 or 140 K-glutamate, 4.7 KCl, 1.13 $MgCl_2$, 10 glucose, 1 EGTA, and 10 HEPES-KOH (pH 7.2). ACh and Ca^{2+} -ionophore A23187 were used to stimulate the acinar cells. Ca^{2+} -ionophore A23187 purchased from Calbiochem-Behring Co., U.S.A., and was dissolved with ethanol, in stock (1 mM). The final concentration of ethanol was less than 0.01% in the test

solution. In some experiments, tetraethylammonium chloride (TEA) (Sigma, U.S.A.) was applied to the bathing solution to test its blocking effect on K^+ channels.

RESULTS

Acetylcholine-evoked whole-cell currents

Figure 1A shows a typical example of the whole-cell current recordings (5 experiments) obtained from a single mouse parotid acinar cell in the standard KCl pipette solution, and the standard NaCl bathing solution. The cell's interior was clamped at -70 mV, with reference to the bathing solution, and 70 mV rectangular depolarizing voltage jumps of 100 ms duration were applied every second. In this way the cell was voltage-clamped alternatively, at the membrane potential of -70 and 0 mV. The addition of 10^{-6} M ACh increased the outward current at 0 mV, but evoked a short-lasting inward current at -70 mV. A few minutes after the start of exposure of the cell to ACh, the inward current at -70 mV declined, and converted to the outward current, and this outward current was more marked at 0 mV. The addition of TEA (5 mM) to the bathing solution had no blocking effect on the ACh-evoked outward current. Both the outward and the inward current evoked by ACh were completely abolished when calcium was removed from the bathing solution, in spite of the continued presence of ACh. The transient inward current was observed before returning to the prestimulating current level of -70 mV. ACh, at a lower concentration (5×10^{-7} M), also caused similar but much smaller responses than those of 10^{-6} M ACh application. In 5 out of 10 cells the effects of 10^{-6} M ACh on the membrane current were completely reproducible, as shown in Fig. 1A.

The whole-cell current-voltage (I - V) relationships, before and during the stimulation with ACh (10^{-6} and 5×10^{-7} M), were shown in Fig. 1B. In the control, I - V relationships showed a marked outward going rectification characteristic of the large K^+ currents. The intersection of the two lines (control and stimulated cell I - V curves) gave an ACh-evoked reversal potential (E_{ACh}) of -74.6 ± 3 mV (mean $\pm$ S.E.; $n=3$). The value of E_{ACh} was slightly positive, with an estimated potassium reversal potential (E_{K}) of -90 mV. Therefore, the current carriers are not only K^+ ions, through the high conductance Ca^{2+} - and voltage-activated K^+ channels (large K^+ channel), but are also Cl^- ions, through the Cl^- channels, and/or Na^+ ions, through the non-selective cation channels.

Ca^{2+} -ionophore A23187-evoked current in Cl^- free solutions

Figure 2 shows the results of an identical experimental protocol to that shown in Fig. 1, except that Cl^- ions in both the pipette KCl and the bath NaCl had been replaced by glutamate and by SO_4^{2-} . When ACh was added to the bathing solution, a marked outward current was observed at 0 mV, but in this

Fig. 1. A: the effects of ACh on whole-cell currents in single mouse parotid acinar cell. The pipette contained a standard pipette solution and the bath contained a standard bathing solution. Holding potential was -70 mV (lower trace in each panel) and the depolarizing voltage jumps of 70 mV (upper trace in each panel) were repetitively applied every second (100 ms duration). The dotted line represents the current level of the holding potential in the control. Outward currents are shown as upwards deflections, and inward currents as downwards deflections. Line Ca free (●), indicates the bathing NaCl solution contained 1 mM-EGTA without addition of Ca^{2+} . Voltage steps of $+60$ to $+90$ mV (10 mV intervals) were applied before ACh application. ACh (10^{-6} M) was present throughout the periods, indicating by line (○), except in the middle trace where concentration of ACh (10^{-6} M) was reduced to 5×10^{-7} M. In the presence of ACh, 10 mV interval voltage steps of $+30$ to $+80$ and -20 to -50 mV (10^{-6} M), $+40$ to $+90$ and -20 to -70 mV (5×10^{-7} M) were applied. Upper, middle, and lower panels were consecutive recordings. Tetraethylammonium (TEA) was added to the bathing solution before the removal of calcium. ↓ indicates the time when the speed of the chart recorder was changed. B: the I - V relationships of the records, shown in Fig. 1A.

Fig. 2. The effects of Ca^{2+} -ionophore A23187 ($0.2 \mu\text{M}$) and ACh ($5 \times 10^{-7} \text{ M}$) on whole-cell currents in single mouse parotid acinar cell. The pipette solution was K-glutamate with 1 mM-EGTA and no added Ca^{2+} . The bath contained a Na_2SO_4 solution with a nominal Ca^{2+} free ($\text{Ca}^{2+} \approx 10 \mu\text{M}$; concentration of Ca^{2+} in this solution was measured by atomic absorption spectroscopy). The holding potential was -60 mV (lower trace) and depolarizing voltage jumps of 60 mV (upper trace) were repetitively applied every second (100 ms duration). The dotted line indicates the current level of the holding potential in the control. Ca free represents the Na_2SO_4 solution contained 1 mM-EGTA without the addition of Ca^{2+} . Depolarization of this cell to 0 mV (upper trace) evoked a large outward current, which was above the maximum recording current (1,000 pA) of the amplifier. ● indicates the current of 1,000 pA.

ionic condition there was no inward current at -70 mV . Application of A23187 caused a marked outward current similar to that evoked by ACh (Fig. 2). MARTY *et al.* (1984) have reported that the effect of A23187 on whole cell currents is quantitatively similar to those of carbamylcholine in the lacrimal gland acinar cells. Thus, A23187 was used instead of ACh in the following experiments since, in many cases (6 of 13 cases), the responses to ACh decreased sharply or was totally absent, presumably as a result of the damaging of the ACh receptors during the cell dissociation with enzymes.

Figure 3B shows $I-V$ relationships in a Cl^- -free solution in the control, and during A23187 application. Voltage jumps from the holding potential of -60 mV to various test potentials were applied first in the control, and then again during the A23187-evoked response (Fig. 3A). Two other examples of $I-V$ curves obtained in this manner are also shown in Fig. 3B. The intersection of the two lines (control and A23187) gave an A23187-evoked reversal potential (E_{A23187}) of $-89 \pm 4 \text{ mV}$ (mean $\pm$ S.E.; $n=3$). The value of E_{A23187} was very close to the estimated K^+ reversal potential (E_{K}) of -90 mV , in this ionic condition.

These results suggest that the major outward current carriers are K^+ ions and Na^+ ions play a small part in ACh- and A23187-evoked responses.

Ca^{2+} -ionophore A23187-evoked current in symmetrical K^+ -free NaCl solutions

If the Cl^- -selective channels are present, A23187-evoked inward current is expected to invert near 0 mV , in zero- Cl^- ionic gradient conditions across the membrane.

A23187-evoked large inward currents were found at a holding potential of

Fig. 3. A: the effects of Ca^{2+} -ionophore A23187 ($0.2 \mu M$) on whole-cell currents in single rat parotid acinar cells. The pipette and the bath solution were the same as Fig. 2, except for the Ca^{2+} concentration in the bath ($1.25 mM$). The holding potential was $-60 mV$ (lower trace), and depolarizing voltage jumps of $60 mV$ lasting $60 msec$ (upper trace) were repetitively applied every second. Depolarizing ($10, 30, 40$, and $50 mV$) and hyperpolarizing ($10, 20, 40$, and $60 mV$) voltage steps were applied during ACh application. Voltage steps of $+60$ to $+100 mV$ ($20 mV$ intervals) were applied before and after ACh application. The dotted line indicates the current level of the holding potential in the control. B: the $I-V$ relationships of the records obtained from Fig. 3A ($\square, \blacksquare$), and from two other experiments ($\circ, \bullet, \triangle, \blacktriangle$). $\times$ indicates the maximum recording current ($1,000 pA$) of the amplifier.

$-60 mV$ (6 experiments), when a K^+ -free NaCl solution was used in both the pipette and the bath, while small outward currents were induced at a membrane potential of $0 mV$, and these were not blocked by TEA (Fig. 4A). $I-V$ curves were constructed from two cases, by measuring the current responses associated with de- and hyperpolarizing voltage jumps in K^+ -free NaCl solutions (Fig. 4B). Intersection between the control and the A23187 curves was taken as E_{A23187} , in a symmetrical NaCl condition. The A23187-evoked currents were reversed at -20

Fig. 4. A: the effect of Ca^{2+} -ionophore A23187 ($0.1 \mu\text{M}$) on the whole cell current in symmetrical K^+ -free NaCl solution in a single rat parotid acinar cell. The pipette solution was NaCl, with 1 mM EGTA, and no added Ca^{2+} . The bath solution contained the same concentration of NaCl as the pipette solution, but 2.5 mM Ca^{2+} and 5 mM TEA were added. The holding potential was -60 mV (lower trace) and depolarizing voltage jumps of 60 mV (upper trace), lasting 60 msec, were repetitively applied. Depolarizing voltage steps of 30 to 80 mV (10 mV intervals) were applied during A23187 application. De- or hyperpolarizing voltage steps of 10 to 90 mV (10 mV intervals) were applied before and after exposure of the cell to A23187. B: the $I-V$ relationships obtained from the record are shown in Fig. 4A (Δ , $\bullet$), and from another record ($\circ$, $\bullet$).

and -12 mV. These values of E_{A23187} were slightly negative, when compared to the calculated Cl^- reversal potential (E_{Cl}) of 0 mV in the symmetrical NaCl ionic gradient across the membrane.

Although the result does not totally exclude, the possibility of the contribution of Na^+ ions to the inward current, the major inward current carriers may be Cl^- ions.

DISCUSSION

The present work directly shows the presence of two types of current (inward and outward currents) evoked by ACh or A23187, in the rat and mouse parotid acinar cells in quasi physiological electrochemical gradient conditions. ACh- or A23187-evoked outward currents would be carried by K^+ efflux through the Ca^{2+} -activated K^+ channels (MARUYAMA *et al.*, 1983a), whereas inward currents may be carried by Cl^- efflux through the Ca^{2+} -activated Cl^- channels (discuss later on). Therefore, when two channels are activated by the agonist, the reversal potential for the agonist-evoked current response must be at potentials between two equilibrium potentials (E_{K} and E_{Cl}), as shown in Fig. 1B. The reversal potential of -63 mV for the ACh-evoked voltage response in mouse parotid gland acinar cells (ROBERTS *et al.*, 1978) could be explained by postulating the agonist-evoked inward and outward currents.

The ACh-evoked inward current at the membrane potential, clamped to the resting potential, could be carried by cation influx or by anion efflux. The Ca^{2+} -dependent non-selective cation channels have been reported in the mouse parotid acinar cells (MARUYAMA *et al.*, 1983a). The non-selective cation channels operate normally in Cl^- -free glutamate or sulphate solutions (MARUYAMA and PETERSEN, 1982). Since there is no inward current response in Cl^- -free conditions (Figs. 2 and 3), and the reversal potential for the A23187-evoked currents (E_{A23187}) in these ionic conditions lies at about -90 mV (Fig. 3B), Na^+ ions through the non-selective cation channels apparently play little part in the overall responses to either ACh or A23187. The value of E_{A23187} in the symmetrical K^+ -free NaCl solution is more positive than that in the presence of K^+ ions (Fig. 4B). It is therefore most likely that the major inward current observed (Figs. 1 and 4) could be carried by Cl^- ions. The values of E_{A23187} (-20 and -12 mV) in the symmetrical NaCl solutions are, however, slightly more negative than the expected value of E_{Cl} (0 mV). We suspect that the difference between the calculated reversal potential and the observed values may be due to the presence of residual K^+ ions in the NaCl dialyzed cells. Very recently, MARTY *et al.* (1984) and FINDLAY and PETERSEN (1985) described inward currents to be carried by Cl^- ions, either during application of cholinergic agonists, or A23187 in the rat and mouse lacrimal gland acinar cells. MARTY *et al.* (1984) have reported the presence of Cl^- channel with a unit conductance of about 1 – 2 pS in the rat lacrimal gland

acinar cells, using stationary noise analysis. It seems reasonable to assume that similar types of Cl^- channel may be responsible for the inward current in the parotid acinar cells, since both cells have very similar responses to ACh (IWATSUKI and PETERSEN, 1978).

The ACh-evoked inward current recorded from parotid acinar cells is usually transient, as shown in Fig. 1A; initially large ACh-evoked inward currents decline rapidly. The mechanisms underlying the rapid decline of the inward current in quasi physiological ionic gradients is not known. The ACh-evoked responses recorded from the lacrimal gland acinar cells are also transient (TRAUTMANN and MARTY, 1984; FINDLAY and PETERSEN, 1985).

In the absence of Cl^- ions in the bath and the pipette solution, the value of E_{A23187} (-89 mV) is very close to the estimated value of E_{K} . ROBERTS *et al.* (1978) have shown that methylsulphate substitution for Cl^- resulted in a shift of E_{ACh} from -63 to -77 mV. Therefore, in the absence of Cl^- , A23187-evoked major current carriers must be K^+ ions through the large K^+ channels, which have been found in the parotid acinar cells (MARUYAMA *et al.*, 1983a).

A23187 directly increases Ca^{2+} or Mg^{2+} fluxes, but not K^+ fluxes across the plasma membrane (REED and LARDY, 1972). Application of A23187, however, results in an increase in the membrane permeability to K^+ ions in rat parotid glands (SELINGER *et al.*, 1974). In the presence of 10 mM EGTA in the pipette, the CCK- or ACh-evoked outward K^+ current is abolished (FINDLAY, 1984; MARUYAMA and PETERSEN, 1984). It is therefore reasonable to assume that ACh and A23187 do not directly affect the K^+ or Cl^- conductance of the parotid acinar cell membrane, instead they act indirectly by increasing intracellular levels of Ca^{2+} . This hypothesis is consistent with the fact that the response to ACh is qualitatively similar to that of A23187 (Fig. 2).

The ACh-evoked sustained responses are totally dependent on the presence of extracellular Ca^{2+} (Fig. 1). This result indicates that calcium influx is necessary to maintain the levels of raised $[\text{Ca}^{2+}]_i$. This has also been suggested in pig pancreatic acinar cells (MARUYAMA and PETERSEN, 1984). Furthermore, conventional microelectrode studies of the mouse pancreatic acinar cells have shown that extracellular Ca^{2+} is necessary for ACh-evoked membrane depolarization (LAUGIER and PETERSEN, 1980; IWATSUKI, 1984). The mechanisms underlying the Ca^{2+} influx are the subject of further investigation.

MARUYAMA and PETERSEN (1984) suggested that the number of K^+ channels per cell (N) increases 2–3 fold with CCK application in the pig pancreatic acinar cells, which has large K^+ channels similar to those in the parotid gland acinar cells (MARUYAMA *et al.*, 1983b). The value of N , estimated by ensemble fluctuation analysis, is about 30–110 (9 experiments), in the unstimulating parotid acinar cells (Maruyama *et al.*, unpublished observations). The N in the parotid acinar cell is not so much different from the values obtained in the lacrimal acinar cells (60–200) (TRAUTMANN and MARTY, 1984) and in the pig pancreatic acinar cells (25–60)

Fig. 5. A: the single channel recordings obtained from the outside-out membrane patch of a mouse parotid acinar cell. The pipette solution was K-glutamate, with 1 mM EGTA, and no added Ca^{2+} . The membrane potential at each trace is written at the left of the individual traces. The current levels, when all channels were closed (0) and one channel was open (1), were indicated. B: the single channel I - V relationships obtained from the records shown in Fig. 5A (●), and from two other experiments (○, ×), are shown.

(MARUYAMA *et al.*, 1983b). It has been shown that the open state probability (P) of the large K^+ channel is regulated by $[\text{Ca}^{2+}]_i$ (MARUYAMA *et al.*, 1983b). ACh or A23187 would therefore, by increasing $[\text{Ca}^{2+}]_i$, be expected to increase P , and this could explain qualitatively the stimulants-evoked increase in the outward current. However, the total K^+ current, which is larger than 1,000 pA at -70 , -60 , and -50 mV, may seem too large to be explained solely in terms of an increase in P (Fig. 6). Assuming that there is one conducting state of the channels (Fig. 5), the total K^+ currents (I_K) could be calculated by the equation of $I = N \cdot P \cdot i$ (i means single channel current amplitude). If this calculation is done using the I - V curves from Fig. 5, and assuming P of 1, it would appear that A23187-evoked total current of 1,000 pA could be only explained by an N of more than 1,000. This explanation cannot, however, be regarded as sufficient evidence for an increase in N , and further work will be required before this question is settled.

The experiments reported here demonstrated the presence of Ca^{2+} -activated Cl^- conducting pathways in the parotid gland acinar cells. PETERSEN and MARUYAMA (1984) have proposed a hypothesis that Ca^{2+} - and the voltage-activated K^+ channel has a crucial role initiating Na-K-Cl cotransport mechanisms on the basolateral membrane in salivary gland acinar cells. The main feature of this hypothesis is that K^+ released through K^+ channels into the intercellular clefts could be taken up again, primarily through a Na-K-Cl cotransport system. Thus,

Fig. 6. The effects of A23187 (●) and ACh (○) on whole-cell current amplitude, at the various holding membrane potentials obtained from rat and mouse parotid single acinar cells. The pipette solution contained either K-glutamate, or K_2SO_4 , and the bathing solution contained Na_2SO_4 . + indicates A23187-evoked current, which was above the maximum recording current (1,000 pA) of the amplifier. The insert traces represent an example that outward-current amplitudes bigger than 1,000 pA (*) at the membrane potentials of -70 and $+10$ mV during A23187 application.

the cycle of K^+ release and K^+ reuptake would be directly linked to the rate of NaCl uptake by the cotransport system. Accordingly Na^+ and Cl^- could be loaded into the cell by these mechanisms, and Na^+ extruded by the electrogenic Na^+ -pump, localized on the basolateral membrane (BUNDGAARD *et al.*, 1977), while Cl^- may be transported into the lumen via Cl^- channels, localized on the luminal membrane. To preserve electroneutrality, Na^+ moves into the lumen through the paracellular pathways. Thus, Ca^{2+} -activated Cl^- conducting pathways could account for an important role for plasma-like NaCl secretion in the parotid acinar cells, if they exists on the luminal membrane. NAUNTOFTE and POULSEN (1985) have shown an existence of the Ca^{2+} -dependent ^{36}Cl transport mechanism in the dispersed rat parotid acinar cells, on which carbachol or A23187 evokes a marked decrease in intracellular ^{36}Cl content.

The role of these Cl^- conducting pathways in electrolyte secretion must remain speculative, until the localization of Cl^- channels is clarified.

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REFERENCES

- BUNDGAARD, M., MØLLER, M., and POULSEN, J. H. (1977) Localization of sodium pump sites in cat salivary glands. *J. Physiol. (Lond.)*, **273**: 339–353.

- FINDLAY, I. (1984) A patch-clamp study of potassium channels and whole cell currents in acinar cells of the mouse lacrimal gland. *J. Physiol. (Lond.)*, **350**: 179–195.
- FINDLAY, I. and PETERSEN, O. H. (1985) Acetylcholine stimulates a Ca^{2+} -dependent Cl^- conductance in mouse lacrimal acinar cells. *Pflügers Arch.*, **403**: 328–330.
- GALLACHER, D. V. and PETERSEN, O. H. (1980) Electrophysiology of mouse parotid acini: Effects of electrical field stimulation and iontophoresis of neurotransmitters. *J. Physiol. (Lond.)*, **305**: 43–57.
- HAMILL, O. P. MARTY, A., NEHER, E., SAKMANN, B., and SIGWORTH, F. J. (1981) Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.*, **391**: 85–100.
- IWATSUKI, N. (1984) Effects of divalent cations on acetylcholine-evoked membrane potential in the ionophore A23187 treated mouse pancreas. *Pflügers Arch.*, **402**: 465–472.
- IWATSUKI, N. and PETERSEN, O. H. (1978) Membrane potential, resistance and intracellular communication in the lacrimal gland: Effects of acetylcholine and adrenaline. *J. Physiol. (Lond.)*, **275**: 507–520.
- LAUGIER, R. and PETERSEN, O. H. (1980) Pancreatic acinar cells: Electrophysiological evidence for stimulant-evoked increase in membrane calcium permeability in the mouse. *J. Physiol. (Lond.)*, **303**: 61–72.
- MARTY, A., TAN, Y. P., and TRAUTMANN, A. (1984) Three types of calcium-dependent channel in rat lacrimal glands. *J. Physiol. (Lond.)*, **357**: 293–325.
- MARUYAMA, Y., GALLACHER, D. V., and PETERSEN, O. H. (1983a) Voltage and Ca^{2+} -activated K^+ channel in basolateral acinar cell membrane of mammalian salivary glands. *Nature*, **302**: 827–829.
- MARUYAMA, Y., PETERSEN, O. H., FLANAGAN, P., and PEARSON, G. T. (1983b) Quantification of Ca^{2+} -activated K^+ channels under hormonal control in pig pancreatic acinar cells. *Nature*, **305**: 228–232.
- MARUYAMA, Y. and PETERSEN, O. H. (1982) Single-channels currents in isolated patches of plasma membrane from basal surface of pancreatic acini. *Nature*, **299**: 159–161.
- MARUYAMA, Y. and PETERSEN, O. H. (1984) Control of K^+ conductance of cholecystokinin and Ca^{2+} in single pancreatic acinar cells studied by the patch-clamp technique. *J. Membr. Biol.*, **79**: 293–300.
- NAUNTOFTE, B. and POULSEN, J. H. (1985) Chloride transport in rat parotid acini: Furosemide-sensitive uptake and calcium-dependent release. *J. Physiol. (Lond.)*, **357**: 61 p.
- PETERSEN, O. H. and MARUYAMA, Y. (1984) Calcium-activated potassium channels and their role in secretion. *Nature*, **307**: 693–696.
- REED, P. W. and LARDY, H. A. (1972) A23187: A divalent cation ionophore. *J. Biol. Chem.*, **247**: 6970–6977.
- ROBERTS, M. L., IWATSUKI, N., and PETERSEN, O. H. (1978) Parotid acinar cells: Ionic dependence of acetylcholine-evoked potential changes. *Pflügers Arch.*, **376**: 159–167.
- SCHNEIDER, L. H., YOUNG, J. A., and SCHNEIDER, C. H. (1972) Salivary secretion of electrolyte. *Physiol. Rev.*, **52**: 720–777.
- SELINGER, Z., EIMERL, S., and SCHRAMM, M. (1974) A calcium ionophore stimulating the action of epinephrine on the adrenergic receptor. *Proc. Natl. Acad. Sci. U.S.A.*, **71**: 128–131.
- TRAUTMANN, A. and MATRY, A. (1984) Activation of Ca -dependent K channels by carbamylcholine in rat lacrimal glands. *Proc. Natl. Acad. Sci. U.S.A.*, **81**: 611–615.
- YOUNG, J. A. and VAN LENNEP, E. W. (1979) Transport in salivary and salt glands. In: *Membrane Transport in Biology*, Vol. 4, Transport Organs, ed. by GIEBISCH, G., Springer, Heidelberg, New York, pp. 280–293.