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PANCREATIC ACINAR CELLS: IONIC DEPENDENCE OF THE MEMBRANE POTENTIAL AND ACETYLCHOLINE-INDUCED DEPOLARIZATION

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SUMMARY

1. Intracellular recordings of membrane potentials have been made *in vitro* from the exocrine acinar cells of the mouse pancreas using glass micro-electrodes.

2. The mean membrane potential of the acinar cells during superfusion with Krebs–Henseleit solution was $-39{\cdot}2$ mV. Increasing $[K]_o$ tenfold decreased the membrane potential by 28 mV when $[K]_o$ was above 10 mM. This depolarization was not affected by atropine ($1{\cdot}4 \times 10^{-6}$ M). Strophanthin-G (10^{-3} M) slowly depolarized the cells at about 10 mV hr^{-1}.

3. Brief exposure to acetylcholine (ACh), $5{\cdot}5 \times 10^{-5}$ M, or pancreozymin resulted in a short lasting depolarization of the acinar cells. Atropine ($1{\cdot}4 \times 10^{-6}$ M) blocked the depolarizing action of ACh but not that of pancreozymin. Adrenaline ($5{\cdot}5 \times 10^{-5}$ M) or cyclic AMP (10^{-3}–10^{-4} M) did not influence the membrane potential.

4. The amplitude of the ACh-induced depolarization was not dependent on the presence of CO_2/HCO_3 in the bathing fluid, but it was closely dependent on the extracellular Na concentration. However, ACh was still able to evoke a small depolarization even after prolonged exposure of the tissue to a Na-free solution.

5. During exposure of the tissue to a Ca-free solution the resting membrane potential was decreased and the ACh-induced depolarization was significantly reduced. Some substances which are known in other tissues to inhibit membrane Ca^{2+} currents, i.e. La^{3+}, D-600 and tetracaine, were able to reduce, but never abolish, the ACh-induced depolarization.

6. These results suggest that the effect of ACh on the pancreatic acinar cell is to increase the permeability of the membrane to commonly occurring ions with a consequent Na-influx and a small Ca-influx.

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INTRODUCTION

The exocrine pancreas has two separate sites for secretion. The acinar cells manufacture enzymes which are released by pancreozymin or vagal nerve stimulation (Harper, 1967) whereas salt and water secretion occurs along the entire length of the duct system in response to stimulation by secretin (Schulz, Yamagata & Weske, 1969). Both electrical stimulation of the pancreatic nerves and pancreozymin have been shown to depolarize the pancreatic acinar cell membrane, whereas secretin was without effect (Dean & Matthews, 1972). In view of the possible importance of this depolarization for enzyme release from the acinar cells, it was decided to investigate the ionic dependence of both the resting membrane potential and the stimulation-induced depolarization. Preliminary accounts of part of this work have already been given (Matthews & Petersen, 1972; Petersen & Matthews, 1972).

METHODS

Membrane potentials were measured in isolated segments of mouse pancreas placed in a Perspex tissue bath (2 ml.) through which a Krebs–Henseleit solution warmed to 37° C was pumped at a constant rate (1·2 ml./min), as described in detail by Dean & Matthews (1972). Micro-electrodes filled with 1·5 M K citrate and having tip resistances between 100 and 200 MΩ were used for impaling the acinar cells. Stimulation of the tissue was normally achieved by adding small quantities of the stimulant directly into the tissue bath. This could be done without disturbance to the intracellular recording.

The standard superfusion solution employed contained (mM): NaCl 103, KCl 4·7, $CaCl_2$ 2·56, $MgCl_2$ 1·13, $NaHCO_3$ 25, NaH_2PO_4 1·15, D-glucose 2·8, Na pyruvate 4·9, Na fumarate 2·7 and Na glutamate 4·9; it was gassed with 95% O_2 and 5% CO_2. At first the substrates pyruvate, fumarate and glutamate were included in the perfusion fluids since this had also been the case in the first successful series of experiments on pancreatic membrane potential measurements (Dean & Matthews, 1970, 1972). Later, attempts were made to omit these three substrates from the perfusion fluids, but the result was always lower membrane potentials and rather few successful impalements.

In some experiments a bicarbonate-free solution was used. The solution was then buffered either by phosphate (3 mM), or Tris (1–5 mM), or both; in these cases the NaCl concentration was augmented to maintain isosmolarity. When a high K concentration was used, NaCl was replaced by an equimolar amount of KCl. When it was desired to reduce the Na concentration, NaCl was replaced by Tris Cl or tetraethylammonium (TEA) Cl. In the cases where a Na-free solution was employed the substrates were added as the acid rather than the Na salt. The pH was always checked and adjusted to 7·4 with concentrated HCl or NaOH when necessary. In the case of the Na-free solution, NaCl and $NaHCO_3$ were replaced by Tris (base) the pH then being adjusted to 7·4 with HCl.

RESULTS

The resting membrane potential

The mean resting membrane potential of 66 ACh-sensitive cells was $-39{\cdot}2$ mV $\pm$ 0·5 S.E. of mean. The dependence of the resting acinar cell membrane potential on $[K]_o$ is shown in Fig. 1. It is clear that above $[K]_o$ 10 mM there is a linear relation between the membrane potential and log $[K]_o$ with a slope of 28 mV per tenfold increase in $[K]_o$. This depolariza-

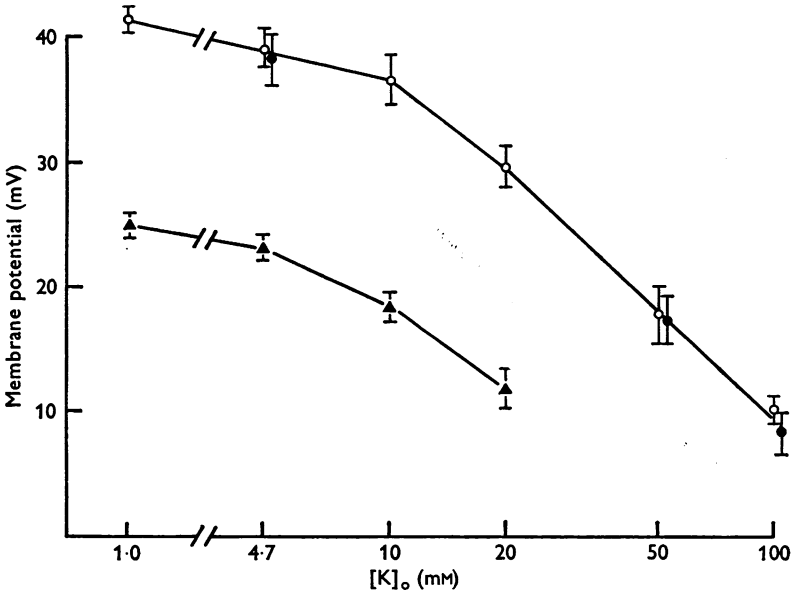

Fig. 1. The dependence of the pancreatic acinar cell membrane potential on the external K concentration. The upper curve represents results of measurements on unstimulated tissue, whereas the lower curve represents results of measurements carried out during superfusion of the tissue with ACh ($5{\cdot}5 \times 10^{-5}$ M). Closed circles indicate results obtained during exposure of the tissue to atropine ($1{\cdot}4 \times 10^{-6}$ M). Mean values (n = 10–80) $\pm$ S.E. of mean are given.

tion, caused by the augmented $[K]_o$, was probably not mediated by release of ACh from depolarized nerve terminals, since it was also seen in the presence of a concentration of atropine ($1{\cdot}4 \times 10^{-6}$ M) sufficient to abolish ACh-induced depolarization (Fig. 5). Reduction of $[K]_o$ from 4·7 to 1 mM failed to cause hyperpolarization.

Strophanthin-G (10^{-3} M) slowly depolarized the cells (10 mV hr^{-1}) as seen in Fig. 2. In one experiment a concentration of Strophanthin-G of 10^{-4} M had no effect on the membrane potential (Fig. 2).

The effect of ACh, pancreozymin, adrenaline and cyclic AMP

During continuous exposure of the tissue to $5{\cdot}5 \times 10^{-5}$ M ACh the membrane potential was decreased. Fig. 3 shows the results from three experiments in which membrane potentials were measured before, during, and after, exposure to ACh. The depolarizing effect of ACh was reversible but the restoration of the membrane potential was slow. Therefore a different mode of stimulation was used routinely, i.e. a single dose of ACh

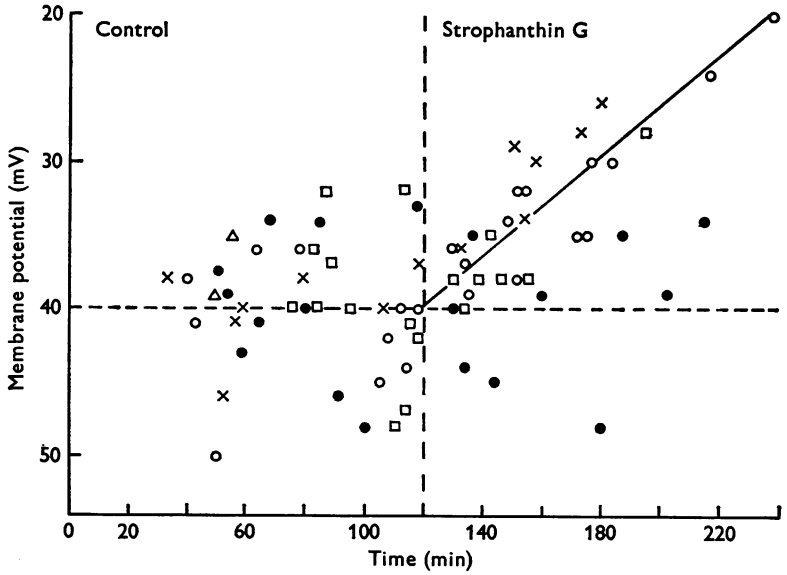

Fig. 2. The effect of Strophanthin-G (10^{-3} M) on the resting membrane potential of pancreatic acinar cells. Results from four preparations are given, each represented by its own symbol. In one of the four preparations the concentration of Strophanthin-G was only 10^{-4} M (●). The continuous line represents the regression line (method of least squares) for the data obtained with the three preparations exposed to Strophanthin-G (10^{-3} M).

was added directly to the tissue bath to achieve the desired concentration for a short period only. In Fig. 4 is shown a typical example of the change in membrane potential following addition of ACh. The response pattern to pancreozymin was indistinguishable from that to ACh. Fig. 5 shows dose–response curves for the depolarizing effect of ACh and pancreozymin. These curves were constructed from experiments of the type shown in Fig. 4. The shape of the curves is little influenced by uncertainty as to the exact concentration of the stimulant drug at the time of maximal depolarization. The depolarization seen in response to an estimated peak concentration of ACh, $5{\cdot}5 \times 10^{-5}$ M, in Fig. 5 is of precisely the same magnitude

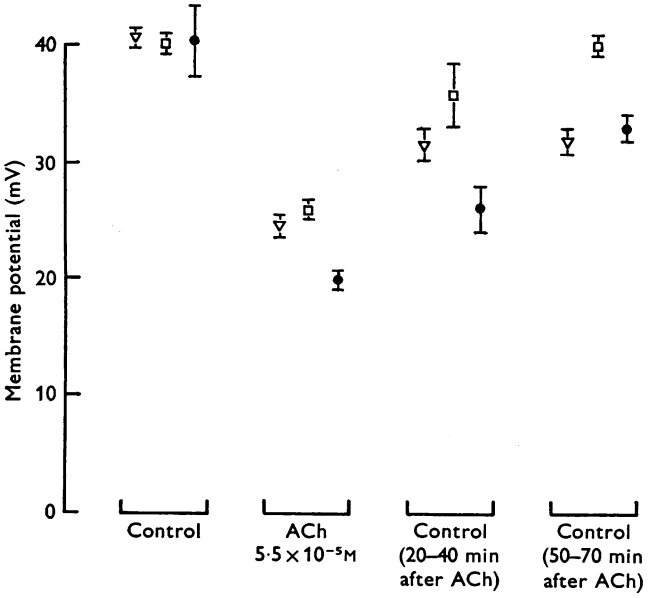

Fig. 3. The effect of superfusion with a Krebs–Henseleit solution containing ACh ($5{\cdot}5 \times 10^{-5}$ M) upon pancreatic acinar cell membrane potential. Mean values (n = 5 to 10) $\pm$S.E. of mean obtained from three preparations, each represented by its own symbol, are given.

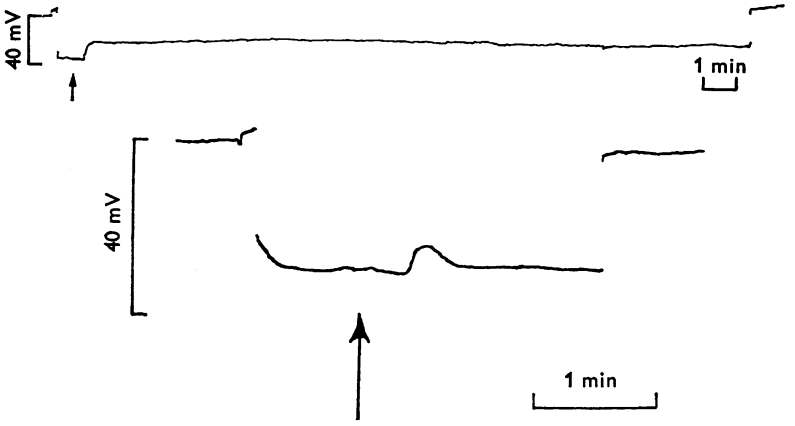

Fig. 4. Intracellular recordings from a pancreatic acinus. A deflexion downwards represents an increased negativity of the micro-electrode. The sudden jump in potential in the left part of the recordings corresponds to the insertion of the micro-electrode into the cell, whereas the sudden shift of the potential to zero in the right part of the tracings corresponds to the retraction of the micro-electrode to the interstitial fluid. The arrows denote addition of ACh to the tissue bath to achieve a peak concentration of approximately $5{\cdot}5 \times 10^{-5}$ M. Upper recording, in normal Krebs–Henseleit solution. Lower recording: another cell from the same preparation impaled 50 min after exposure to tetracaine, 10^{-3} M.

as that seen in response to the same concentration maintained for the longer period shown in Fig. 3. This is consistent with the assumption that the estimated concentrations in Fig. 5 are correct or too high, but not too low. The depolarizing effect of ACh but not that of pancreozymin, was abolished by atropine ($1{\cdot}4 \times 10^{-6}$ M). Adrenaline or noradrenaline ($5{\cdot}5 \times 10^{-5}$ M) had no effect on the acinar cell membrane potential and had also no effect on the ACh-induced depolarization in three preparations in which

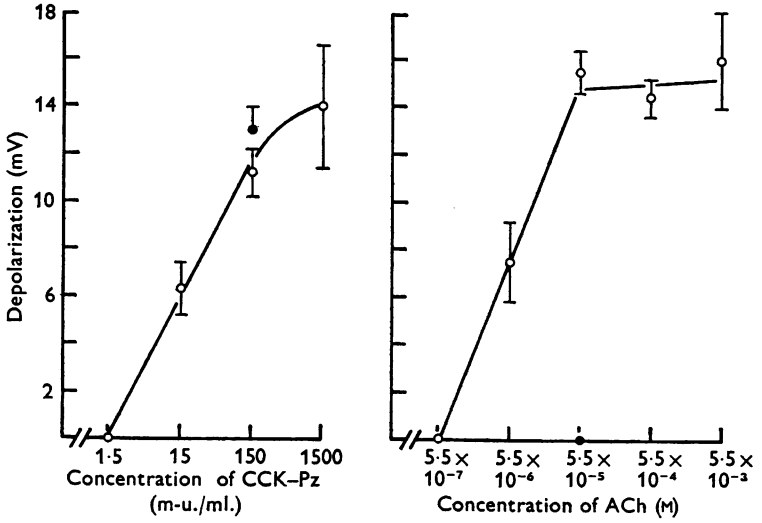

Fig. 5. Dose–response curves for the depolarizing effect of ACh and pancreozymin on the pancreatic acinar cells. Mean values (n = 5–73) ± S.E. of mean are given. The filled circles represent results of measurements carried out during exposure of the tissue to atropine ($1{\cdot}4 \times 10^{-6}$ M). Pancreozymin concentrations expressed in Crick–Harper–Raper units.

it was tested. Adenosine 3′,5′-cyclic monophosphate (cyclic AMP) or dibutyryl cyclic AMP (10^{-4}–10^{-3} M) likewise had no effect on the membrane potential or ACh-induced depolarization when tested in two preparations.

The importance of extracellular CO_2/HCO_3

It is known that the presence of extracellular CO_2/HCO_3 is necessary for the secretion of pancreatic juice (Case, Scratcherd & Wynne, 1970). It was therefore of interest to determine the relative importance of CO_2/HCO_3 for the maintenance of the acinar membrane potential. During superfusion of the tissue with a CO_2-free phosphate buffered solution the resting membrane potential was $-35{\cdot}4$ mV ± 1·0 S.E. of mean (13) and in these cells ACh caused depolarizing secretory potentials with a mean amplitude of 14·6 mV ± 1·2 S.E. of mean. If Tris buffer rather than phosphate buffer

was used to replace the CO_2/HCO_3 buffer the mean RP was $-34{\cdot}5$ mV $\pm$ 1·0 S.E. of mean (18) and the mean ACh-induced depolarization 15·4 mV $\pm$ 0·8. This compared with the mean resting potential of $-39{\cdot}2$ mV $\pm$ 0·5 S.E. of mean recorded in Krebs–Henseleit solution containing CO_2/HCO_3, where the mean amplitude of the ACh-induced depolarization was 15·5 mV $\pm$ 0·9 S.E. of mean.

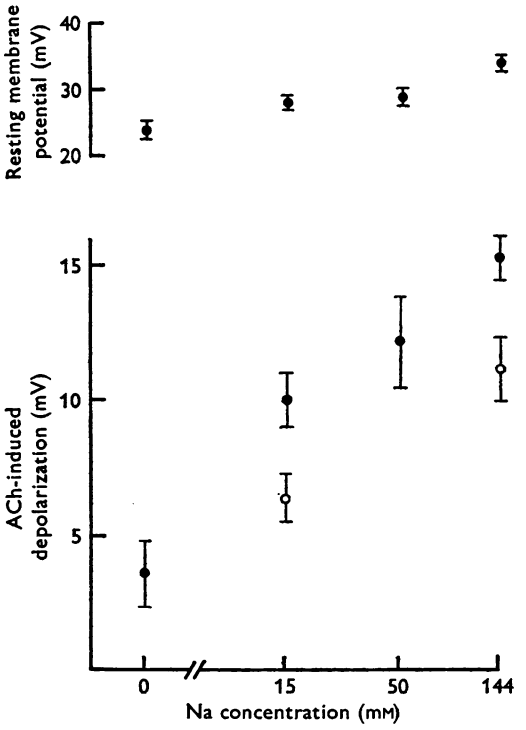

Fig. 6. The dependence of the resting membrane potential and the ACh-induced depolarization on the superfusion fluid Na concentration. The stimulation was carried out by adding ACh directly to the tissue bath to achieve a peak concentration of $5{\cdot}5 \times 10^{-5}$ M. For comparison (○), results obtained with pancreozymin stimulation (150 m-u./ml., Crick–Harper–Raper units) at two different Na concentrations. Mean values (n = 11–20) $\pm$ S.E. of mean are given.

The importance of extracellular Na

As seen in Fig. 6 the mean amplitude of the ACh-induced depolarization was closely dependent on the extracellular Na concentration. However, even during exposure of the tissue to a completely Na-free solution the depolarization in response to ACh stimulation was not abolished. As late as 90 min after the start of superfusion with a Na-free solution small

depolarizations in response to ACh were seen. The reduction in the amplitude of the ACh-induced depolarization could, however, be caused by the decreased resting membrane potential *per se*. The results presented in Fig. 6 were therefore compared with those from another series of experiments in which membrane depolarization was achieved by means other than simply reducing the extracellular Na concentration. Fourteen cells impaled during exposure of the pancreatic tissue to amiloride (10^{-4} M), Strophanthin-G (10^{-3} M), 2,4-dinitrophenol (10^{-4} M) or a solution containing $[K]_0$ = 20 mM, selected because the membrane potential ranged between −20 and −30 mV (mean value −24·5 mV ± 0·8 S.E. of mean); still responded to ACh with a mean depolarization of 13·9 mV ± 0·9 S.E. of mean. It would therefore appear that the reduction in the amplitude of the ACh-induced depolarization seen during exposure to a solution containing subnormal amounts of Na is caused directly by the low $[Na]_0$ rather than by the reduced resting membrane potential *per se*.

The importance of extracellular K

The response of the ACh stimulated acinar cell membrane to variations in extracellular K concentration did not differ markedly from that of the resting cell membrane (Fig. 1). However, some small effects of varying the extracellular K concentration on the mean amplitude of the ACh-induced depolarization were noted. At $[K]_0$ = 4·7 the mean amplitude of the ACh-induced depolarization was 15·4 mV ± 0·8 S.E. of mean. At $[K]_0$ = 10 and 20 mM the comparable figures were 18·5 mV ± 1·5 and 17·8 ± 1·6 respectively. Although the increments are statistically non-significant, some effect was distinguishable in individual experiments. On the other hand simply reducing $[K]_0$ from 4·7 to 1 mM did not reduce the mean amplitude of the ACh-induced depolarization (16·3 mV ± 0·8 S.E. of mean).

The importance of Ca

During exposure of the pancreatic tissue to a Ca-free solution the resting membrane potential was markedly decreased, whereas the mean amplitude of the ACh-induced depolarization was only slightly, though significantly smaller ($P < 0·05$) than during control conditions (Fig. 7). In one preparation the resting membrane potential 150 min after exposing the tissue to Ca-free solution was 14 mV, yet this cell still responded to addition of ACh to the tissue bath with a depolarization of 10 mV. D-600 (α-isopropyl-α-(*N*-methyl-*N*-homoveratryl)-γ-aminopropyl-3,4,5-trimethoxy-phenylacetonitril) (10^{-4} M), tetracaine (10^{-3} M) and La^{3+} (5 mM), substances that interfere with membrane Ca^{2+} currents in other tissues (Kohlhardt, Bauer, Krause & Fleckenstein, 1972; Douglas & Kanno, 1967; Van Breemen & DeWeer, 1970) all caused some reduction in the mean amplitude of the

ACh-induced depolarization (Fig. 7). Since the effect of tetracaine on the ACh-induced depolarization might be due to some interaction with the muscarinic receptor (Ritchie & Greengard, 1966) the effect of tetracaine on the pancreozymin-induced depolarization was also tested. In control conditions pancreozymin caused a mean depolarization of 13·8 mV ± 1·4 S.E. of mean; in the presence of tetracaine (10^{-3} M) the depolarizing effect was reduced to 6·0 mV ± 1·9 S.E. of mean. Apart from the effect of tetracaine on the amplitude of the ACh-induced depolarization a considerable reduction of the duration of the ACh-induced change in membrane potential was also noted in some preparations (Fig. 4).

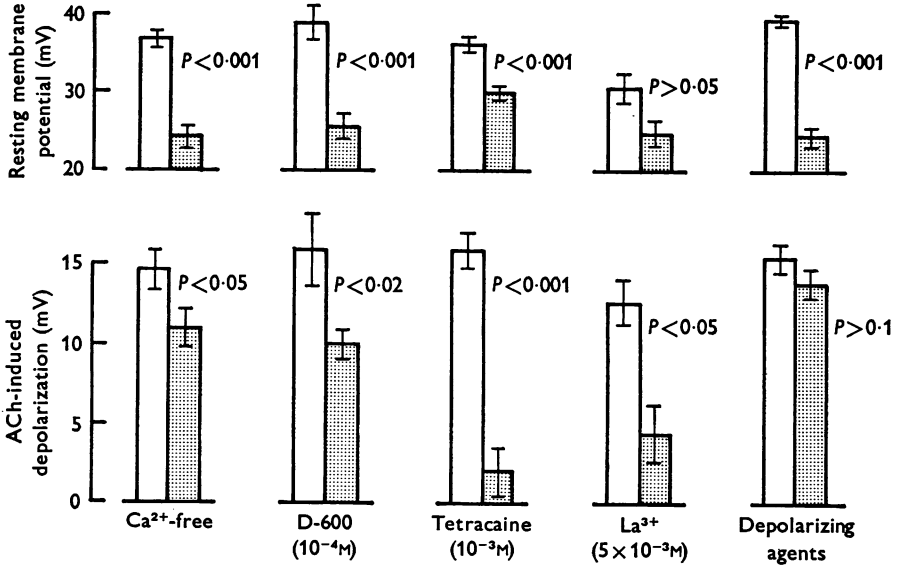

Fig. 7. The importance of extracellular Ca for the resting membrane potential and the ACh-induced depolarization of pancreatic acinar cells. The open columns represent control measurements from the same preparations as the test results. The control data were obtained during exposure of the tissue to a normal Krebs–Henseleit solution except in the La^{3+} series where a Tris buffer (1 mM) rather than CO_2/HCO_3 was employed. The test data in the last series denoted depolarizing agents were derived from tissues exposed to amiloride (10^{-4} M), Strophanthin-G (10^{-3} M), 2,4-dinitrophenol (10^{-4} M) or a solution containing K in a concentration of 20 mM. ACh was used in a concentration of $5{\cdot}5 \times 10^{-5}$ M.

DISCUSSION

The resting membrane potential

The mean value of the resting acinar membrane potential (RP) from the *in vitro* mouse pancreas of about −40 mV is very close to the value

obtained by Dean & Matthews (1972) from the same preparation, but rather higher than the value of −35 mV reported by Kanno (1972) in studies on the perfused rat pancreas. There is agreement between our results and those of Kanno in that variations in the extracellular K concentration, $[K]_o$, markedly influence the membrane potential (Fig. 1). The importance of the K concentration gradient across the cell membrane for the membrane potential is further demonstrated by the results obtained with Strophanthin-G (10^{-3} M), since this drug slowly depolarized the acinar cells. It is likely that at this concentration the inhibitor arrests the Na-K pump and causes a loss of intracellular K, thereby reducing the transmembrane K concentration gradient. There exists only one direct measurement of the intracellular K and Na concentrations in the mouse pancreas (Morrill, Kabach & Robbins, 1964). In this study $[K]_i$ was about 108 and $[Na]_i$ 71 m-mole/l. cell water. The $[K]_i$ value is surprisingly low whereas the reverse is true for $[Na]_i$. Schneyer & Schneyer (1960) found $[K]_i$ to be 155·5 and $[Na]_i$, 18·5 m-mole/kg cell water in the rat pancreas. If one extrapolates to zero membrane potential the curve in Fig. 1, relating RP to log $[K]_o$, the intercept gives $[K]_o$ = about 200 m-mole/l. This value should theoretically be equal to $[K]_i$. The K equilibrium potential (E_K) is equal to or more negative than −77 mV (calculated on the basis of the data of Morrill *et al.* 1964) and thus the RP is undoubtedly much less negative than E_K, suggesting a marked permeability to at least one other ion having an equilibrium potential which is more positive than −40 mV. The resting acinar cell membrane, in addition to having a high K permeability, could be relatively permeable to Na as may be the case generally for non-excitable cells (Williams, 1970), thus explaining why the RP is lower than E_K. In many gland cells it has been shown that reduction of $[Ca]_o$ causes depolarization (Douglas, Kanno & Sampson, 1967; Petersen, Poulsen & Thorn, 1967; Dean & Matthews, 1970) and this was also found in the present study (Fig. 7). Surprisingly Kanno (1972), on the rat exocrine pancreas, got the opposite result.

The effect of ACh and pancreozymin

As shown in Fig. 5 both ACh and pancreozymin depolarized the cells in a dose-dependent fashion. The depolarizing effect was reversible (Fig. 3 and 4). The mean value of the depolarization obtained with a maximal stimulus was about 15 mV which corresponds closely to the values given by Dean & Matthews (1972) for the depolarizing effect of acetyl-β-methylcholine, pancreozymin or maximal nerve stimulation. Kanno (1972) on the other hand states that both ACh and pancreozymin hyperpolarize the rat pancreas acinar cell membrane by about 4–5 mV. Kanno has, however, not shown this hyperpolarizing effect during continuous intracellular

recording from one cell. In our experiments the depolarizing effect of ACh, but not that of pancreozymin, was abolished by atropine (Fig. 5), suggesting the existence of two distinct membrane receptors for these agonists.

It has been claimed that cyclic AMP stimulates enzyme secretion from the pancreas (Kulka & Sternlicht, 1968; Ridderstap & Bonting, 1969), although other investigations have not supported this view (Case & Scratcherd, 1972; Benz, Eckstein, Matthews & Williams, 1972). It is therefore of interest to note that neither cyclic AMP nor dibutyryl cyclic AMP (10^{-3} M) had any effect on the membrane potential.

Ionic mechanisms of stimulant-induced depolarization

The amplitude of the ACh-induced depolarization is clearly dependent on $[Na]_o$ (Fig. 6). However, during exposure of the tissue to a Na-free solution small depolarizations in response to ACh could still be evoked. These findings are essentially similar to those obtained on the adrenal medulla, where Douglas *et al.* (1967) showed that depolarization in response to ACh was Na-dependent but not abolished in Na-free media. Douglas *et al.* (1967) were also able to show that the small depolarization seen in response to ACh in Na-free media was closely dependent on $[Ca]_o$ and could be abolished by the local anaesthetic tetracaine (Douglas & Kanno, 1967). This drug also reduced the ACh-induced depolarization during exposure of adrenal medullary cells to a normal Na-containing medium. In the present study it has been shown that the amplitude of the ACh-induced depolarization is somewhat reduced during exposure to Ca-free solutions and also that the local anaesthetic tetracaine reduces severely both the ACh and pancreozymin induced depolarization. Furthermore, D-600 and La^{3+} both reduced the amplitude of the ACh-induced depolarization (Fig. 7). These findings taken together suggest that in addition to Na-influx Ca-influx is of importance in mediating the ACh-induced depolarization. The available data suggest that the Na current normally predominates. This fits into the picture we now have of the effect of ACh on several cell membranes. In the motor end-plate the depolarizing effect of ACh is due mainly to Na entry (Fatt & Katz, 1952; Takeuchi & Takeuchi, 1960) and as already mentioned this is also the case in the adrenal medulla (Douglas *et al.* 1967). In the acinar cells of the salivary glands the main effect of ACh seems to be to evoke Na-influx and K-efflux (Petersen, 1970*a*, *b*).

ACh and pancreozymin stimulation causes protein release from the acinar cells by the process of exocytosis (Palade, 1959). In the model proposed by Douglas (1968) for the control of exocytosis in a variety of secretory cells, the stimulating agent is believed to cause an increase in membrane permeability to Na and Ca with a resulting cation influx and

depolarization. Ca-influx is essential for initiation of the secretory process, perhaps due to an interaction with the granule/cell membrane (Matthews, 1970). Thus it may well be Ca-influx *per se*, rather than the more dominant Na-influx, which is the essential event in stimulus-secretion coupling in the exocrine pancreas.

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