ELECTRICAL COUPLING AND UNCOUPLING OF EXOCRINE ACINAR CELLS

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ABSTRACT

The electrical communication network in the mouse pancreatic acinar tissue has been investigated using simultaneous intracellular recording with two separate microelectrodes and direct microscopical control of the localizations of the microelectrode tips.

All cells within one acinus were electrically coupled, and the coupling coefficient (the electrotonic potential change in a cell neighboring to the cell into which current is injected [V_2] divided by the electrotonic potential change in the cell of current injection [V_1]) between two cells near each other (<50 μm) was always close to 1. Cells farther apart (50–100 μm) were, in some cases, coupled; in other cases, there was no coupling at all. Coupling coefficients varied between 0 and 1. There was rarely electrical coupling over distances of more than 110 μm.

Using microiontophoretic acetylcholine (ACh) application, it was possible to evoke almost complete electrical uncoupling of two previously coupled pancreatic or lacrimal acinar cells from different acini or within one acinus. The effects were fully and quickly reversible. While the ACh-evoked uncoupling in the pancreas was associated with membrane depolarization, ACh caused hyperpolarization in the lacrimal acinar cells. The uncoupling was associated with a very marked reduction in electrical time constant, indicating a reduction in input capacitance (effective surface cell membrane area).

The concentrations of stimulants needed to evoke reduction in pancreatic cell-to-cell coupling were 1 μM for ACh, 0.14 nM for caerulein, and 3 nM for bombesin. These concentrations are smaller than those required to evoke maximal enzyme secretion.

KEY WORDS exocrine pancreas · exorbital lacrimal gland · stimulant evoked electrical uncoupling · acetylcholine · caerulein

Electrical communication between neighboring cells has been described in a large number of different tissues (11, 25). There is extensive, though indirect, evidence showing that direct intercellular communication occurs through channels located in the gap junctions (1, 12, 30, 33, 36).

Electrical communication between pancreatic acinar cells has been demonstrated (31), and pancreatic tissue contains large gap junctions (7). In the liver, which also contains extensive gap junctions (7, 14), a very wide-ranging electrical

coupling has been demonstrated (15, 28). In contrast, coupling in pancreatic acinar tissue seems to be restricted to a relatively small domain (31) similar to what has been shown for the thyroid gland (22). So far, a systematic mapping of voltage fields in mammalian tissues with restricted coupling, as found in the thyroid (22), the pancreas (31) and the lacrimal gland (20), has not been undertaken.

It has recently been shown that stimulation of the mouse or rat pancreas with relatively high hormone concentrations can evoke a fully reversible decrease in ionic coupling between acinar cells (19, 21). This finding raises several questions: (*a*) Is it possible to evoke complete or nearly complete electrical uncoupling between acinar cells that are fully coupled in the resting state? (*b*) Would such a complete stimulant-evoked uncoupling still be rapidly reversible? (*c*) Can hormones in physiological concentrations cause a decrease in electrical coupling between acinar cells? And (*d*) can a stimulant-evoked decrease in electrical coupling between neighboring acinar cells also be demonstrated in exocrine glands other than the pancreas?

The aim of the present work was to map the electrical communication network in the pancreatic acinar tissue, to describe the basic electrical unit, to quantify the uncoupling action of secretagogues with regard to changes in electrical unit size, to establish the minimum concentrations of secretagogues necessary for uncoupling, and to compare secretagogue-evoked uncoupling in the pancreas with the lacrimal gland.

MATERIALS AND METHODS

Pancreases or exorbital lacrimal glands were removed from mice, and small segments (2 × 2 mm) were placed on a translucent perspex platform in a bath (16 ml) through which a physiological salt solution was circulated (20 ml/min). The solution 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; Na glutamate, 2.7; and was gassed with 95% O_2, 5% CO_2. Under visual control (Zeiss phase-contrast microscope, × 640, 2-cm working distance) two microelectrodes were inserted into different acinar cells (Figs. 1 and 2). The glass microelectrodes were filled with 3 M KCl by the fibre glass method, were beveled on a K. T. Brown beveler (Sutter Instrument Co., San Francisco, Calif.), and had final resistances of 30–50 MΩ. Membrane potentials and resistances were measured with the help of electrometer amplifiers (M701, M750, W-P Instruments, Inc., New Haven, Conn.) as previously described (18, 31). The potentials were displayed on a storage oscilloscope and a pen recorder. An extracellularly located third micropipette filled with 2 M AChCl was used for iontophoretic stimulation of impaled acini. The iontophoretic current was measured using a virtual ground current-voltage converter (M180, W-P Instruments, Inc.). Photographs were taken through the phase-contrast microscope and from the oscilloscope screen. In some cases, secretagogues were included in the superfusion solution in known concentrations. AChCl was purchased from BDH while synthetic caerulein (caeruletide) and bombesin were gifts from Dr. R. de Castiglione (Farmitalia, Ricercha Chimica, Milan, Italy).

RESULTS

The mouse pancreas is a thin (<200 μm) translucent organ. At the edges of the preparation, it was in most cases possible to identify individual cells and select them for impalement (Fig. 1).

Acinar Cells Coupled with a Coupling Coefficient of 1

When two microelectrodes were inserted into neighboring cells (Fig. 2) and current pulses were passed through one of the electrodes, electrotonic potential changes were observed not only in the cell into which current was injected but also in the neighboring cell (31) (Fig. 3, A). The amplitude and the time course of the electrotonic potential change in the cell of current injection (V_1) was in such cases indistinguishable from the electrotonic potential change in the neighboring cell (V_2) (Fig. 4). The coupling coefficient (V_2/V_1) was therefore 1. When the tips of the two microelectrodes were a few cells apart, coupling coefficients of 1 were still inevitably found. Even at relatively long distances (50–70 μm), coupling coefficients of 1 were often observed.

All acinar cells impaled responded to short pulses (0.5 s) of ACh stimulation with depolarization and resistance reduction (18). In two fully coupled cells (Fig. 3), the depolarizations and resistance reductions were alike in magnitude as well as time course. The resistance reduction was associated with a marked reduction of the electrical time constant (Fig. 4). The time course of the rising phase of the electrotonic potential changes (V_1 or V_2) could always be fitted to an equation of the type $V = RI(1 - e^{-t/RC})$ where R is the input resistance, I the injected current, and C the input

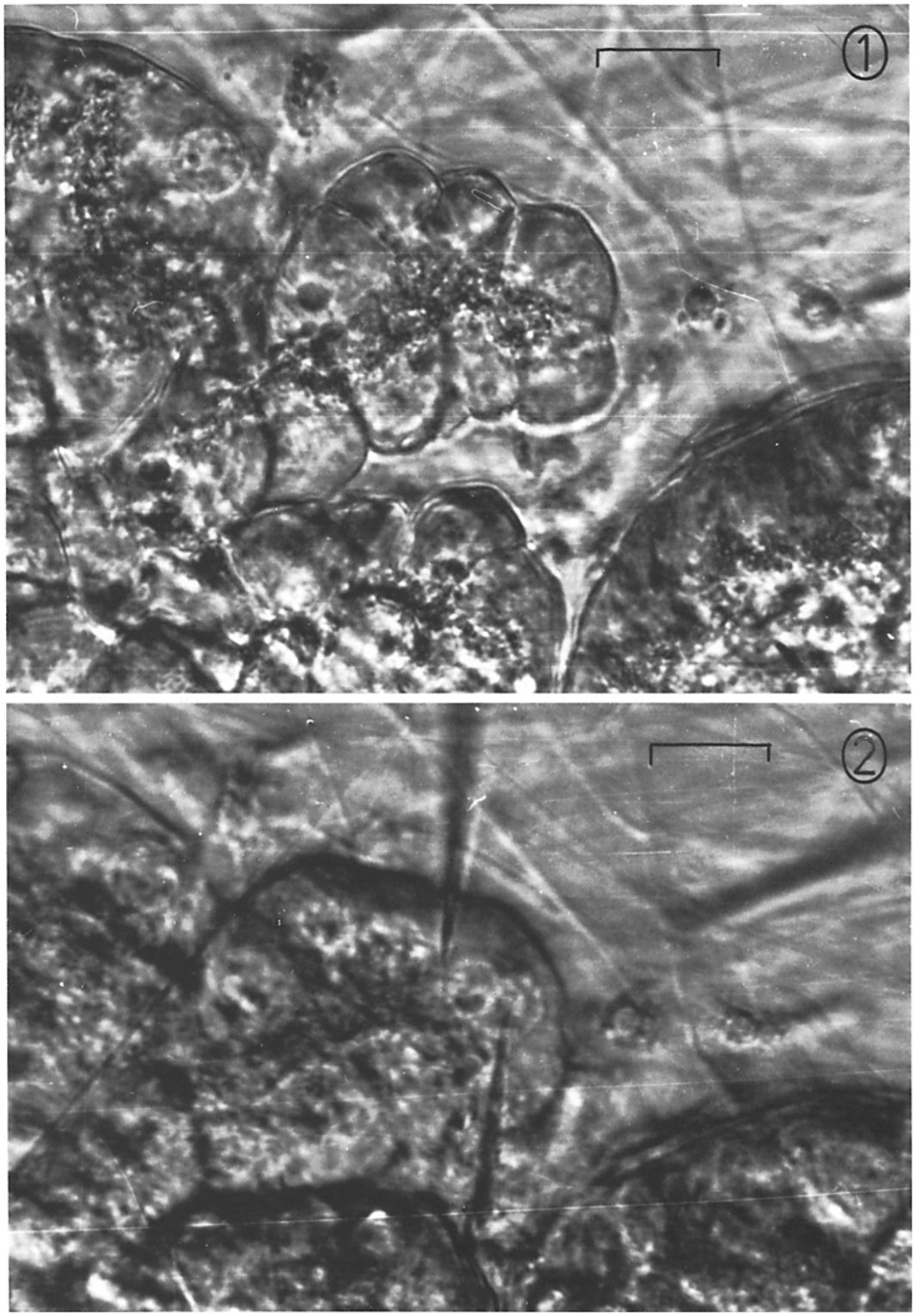

FIGURE 1 Photograph, taken through phase-contrast microscope, of the edge of mouse pancreas fragment placed in the perspex bath. Bar, 20 μm. × 900.

FIGURE 2 Same field as in Fig. 1, but now two microelectrodes have been inserted into two neighboring cells. The tip of the extracellular AChCl-filled micropipette is also seen. Bar, 20 μm., × 900.

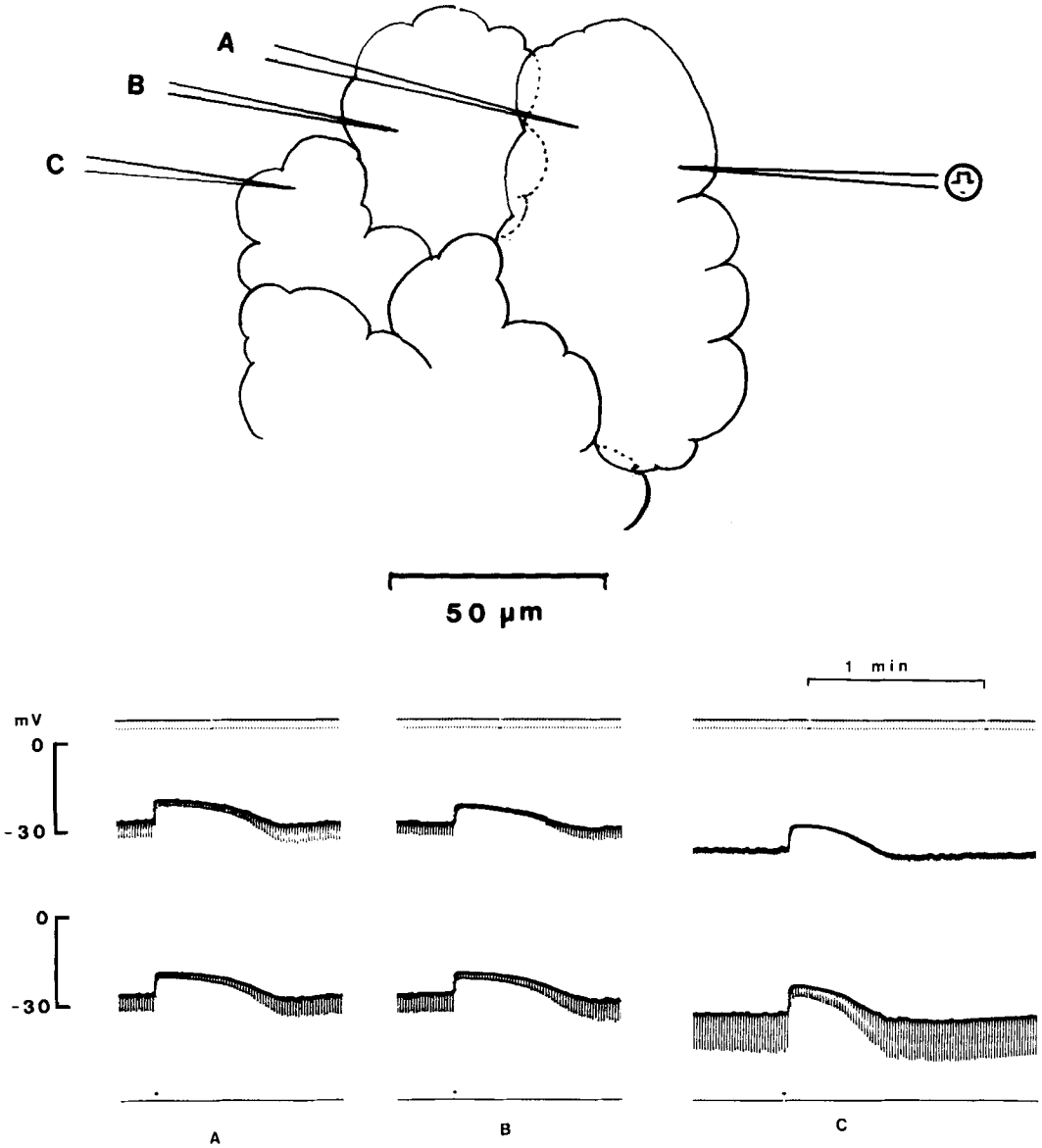

FIGURE 3 Membrane potential and resistance measurements in three situations. The traces shown in the first section of the pen recording (*A*) were obtained with the current-injection electrode (⎍⎍) in the position as indicated in the schematic diagram and electrode A. The traces shown in the second panel (*B*) were obtained with the current-injection electrode (⎍⎍) in the same position but with the second electrode in position B, etc. The current-injection electrode always measured the potentials shown in the lower of the two membrane potential traces. In sections *A* and *B* intracellular current pulses were of 2 nA intensity while in *C* they had a value of 4 nA. At marker signals (bottom traces), ejecting current pulses were applied to the ACh-containing micropipette (60 nA, 0.5 s; retaining current: 20 nA). The uppermost trace in the pen recording is a time marker trace.

capacitance. In the case shown in Fig. 4, the electrotonic potential changes (V_1 or V_2) during the resting condition could be fitted by the equation $V = 9(mV)(1 - e^{-t(ms)/15(ms)})$. During the action of ACh, V_1 or V_2 could be fitted by $V = 2(1 - e^{-t/3})$. During rest the input resistance was

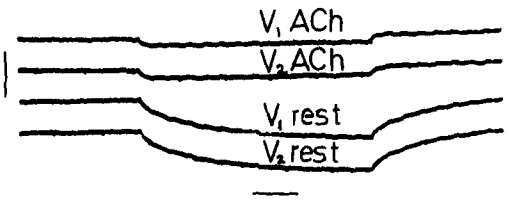

FIGURE 4 The time course of the electrotonic potential changes in two closely coupled cells within the same acinus in response to rectangular hyperpolarizing currents (2 nA, 100 ms). V_1 denotes potential in current injection cell while V_2 represents potential in neighboring cell. The resting potential of both cells was −40 mV. V_1 and V_2 ACh were obtained a few seconds after application of ejecting current to ACh-containing pipette (80 nA, 0.5 s, retaining current: 20 nA). Calibration: horizontal, 20 ms; vertical, 10 mV.

4.5 MΩ, but during the action of ACh the input resistance was reduced to 1 MΩ. The time constant RC was therefore reduced by the action of ACh in proportion to the reduction in resistance, indicating that the input capacitance (effective surface cell membrane area) was constant.

Acinar Cells Coupled with Coupling Coefficients <1

Electrical coupling between different cells was sometimes associated with coupling coefficients smaller than 1 (Fig. 3, B). In such cases, the tips of the impaling microelectrodes were found mostly in what appeared to be different acini. Stimulation with short pulses of ACh, in such instances, caused a decrease in the coupling coefficient. In the experiment shown in Fig. 3, B, the coupling coefficient in the resting state was ~0.7. At the height of stimulation, a value of 0.5 was found. The effect of ACh was fully reversible.

Acinar Cells not Electrically Coupled

Cells from different acini not far from each other were frequently not coupled at all (Figs. 3, [C] and 5). In such cases, there were often quantitative differences in the amplitude and time course of ACh-evoked depolarizations. The depolarization evoked by ACh in the case shown in Fig. 5 was 21 mV in one acinus (upper trace) but only 17 mV in the other acinus (lower trace). It was also possible to observe spontaneous miniature depolarizations, because of spontaneous ACh release from nerve endings (4, 27), in one acinus which were not transmitted to the other (Fig. 5).

Coupling Coefficient as a Function of Distance between Cells

Fig. 6 displays the combined data obtained from this series of experiments. It is seen that, up to distances of 75 μm, coupling coefficients of 1 were frequently found. At distances of >50 μm, totally uncoupled cell pairs were found, and at distances of >75 μm, coupling coefficients of 0 were most frequently obtained. High coupling coefficients occurring over distances of up to 110 μm were observed in a few cases.

ACh-Evoked Reduction in Pancreatic Acinar Cell-to-Cell Coupling

When cells some distance apart (50–100 μm) (in two neighboring acini) were impaled, electrical coupling was, as already described, frequently observed. A short pulse (1 s) of ACh stimulation caused depolarization and reduction of surface membrane resistance and, as seen in Fig. 7, a reduction in coupling coefficient (V_2/V_1). This finding does not necessarily imply that a change in junctional resistance has taken place; on the other hand, it does not exclude this possibility. Longer pulses of ACh stimulation (10 s) caused clear uncoupling (Fig. 7). The electrotonic potential changes in the cell into which current pulses of constant size were injected (V_1) increased markedly, after the initial decrease, while the electrotonic potential changes in the other cell (V_2) almost disappeared (Fig. 7). At the height of the response, V_1 was larger than in the resting state, but in spite of this the electrical time constant had been reduced. The time course of the ascending phase of V_1 in the resting state fits the curve $V = 9.5(1 - e^{-t/RC})$, where RC = 20 ms. The input resistance R is, in this case, 9.5 mV/2 nA ≈ 4.8 MΩ. C can therefore be calculated to have a value of 4.2 nF. At the height of uncoupling, the time course of V_1 fits the curve $V = 17(1 - e^{-t/RC})$, where RC = 2 ms. The input resistance R is, in this case, 17 mV/2 nA = 8.5 MΩ. C therefore has a value of 0.24 nF. At constant capacity per cm² surface cell membrane (24), this corresponds to an 18-fold reduction in the effective surface area of this electrical unit.

The most important criterion for an increase in junctional resistance is the rise in amplitude of the electrotonic potential in the cell into which current pulses are injected. When ACh pulses were given

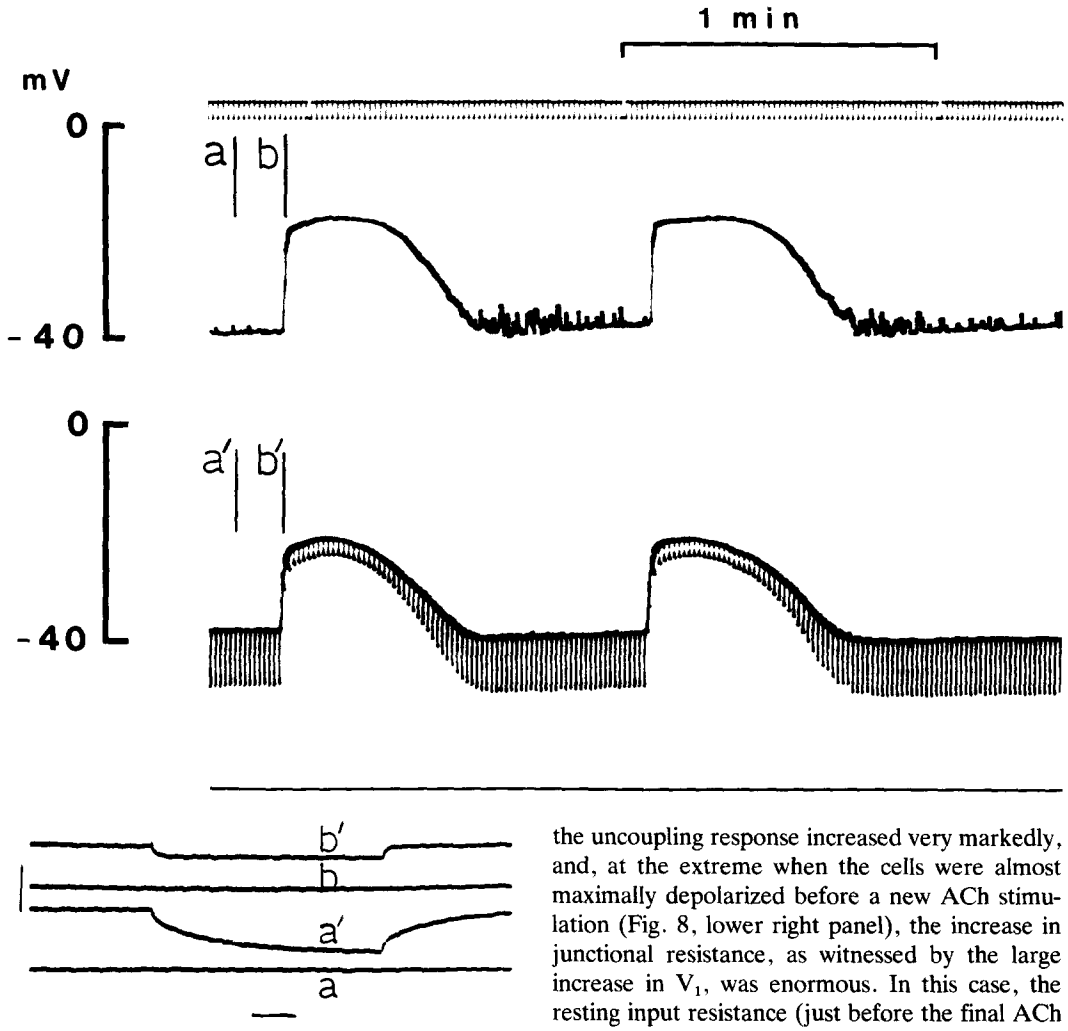

FIGURE 5 Membrane potential and resistance recordings from two cells in two different acini. Distance between microelectrode tips: 60 μm. The intracellular current injection (2 nA, 100 ms) electrode is the one recording the potential shown in the lower membrane potential (pen recording) trace. At marker signals (bottom trace); ejecting current pulses of 80 nA were applied for 0.5 s to the ACh micropipette. The oscilloscope photograph shows the time course of the electrotonic potential changes. Calibration: horizontal, 20 ms; vertical, 10 mV.

at such short intervals that the cells could not recover completely before the next stimulation period, a progressive depolarization and reduction in surface cell membrane resistance was observed (Fig. 8). For each subsequent larger ACh dose, the uncoupling response increased very markedly, and, at the extreme when the cells were almost maximally depolarized before a new ACh stimulation (Fig. 8, lower right panel), the increase in junctional resistance, as witnessed by the large increase in V_1, was enormous. In this case, the resting input resistance (just before the final ACh application) was 1.3 MΩ and the coupling coefficient was 0.7. The minimum input resistance immediately after ACh application was 0.8 MΩ, but the input resistance then rose to a maximum value of 55 MΩ. At that point (the height of uncoupling) the coupling coefficient was 0.008. There was, in this instance, a 65-fold increase in input resistance because of the ACh-evoked increase in junctional resistance (Fig. 8, lower right panel).

Using microiontophoretic ACh application, it is not possible to calculate very accurately the ACh concentration achieved near the receptor sites. To get information on the ACh concentrations needed to evoke increases in junctional resistance, pancreatic segments were superfused with solutions containing known concentrations of ACh. In

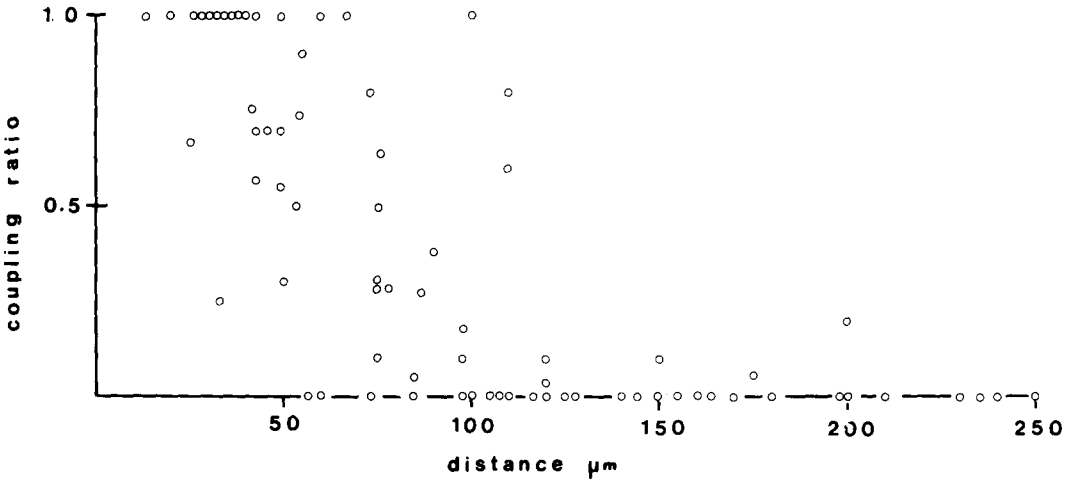

FIGURE 6 The coupling ratio (coupling coefficient) (V_2/V_1) between two cells in the pancreatic acinar tissue as a function of the distance between the cells.

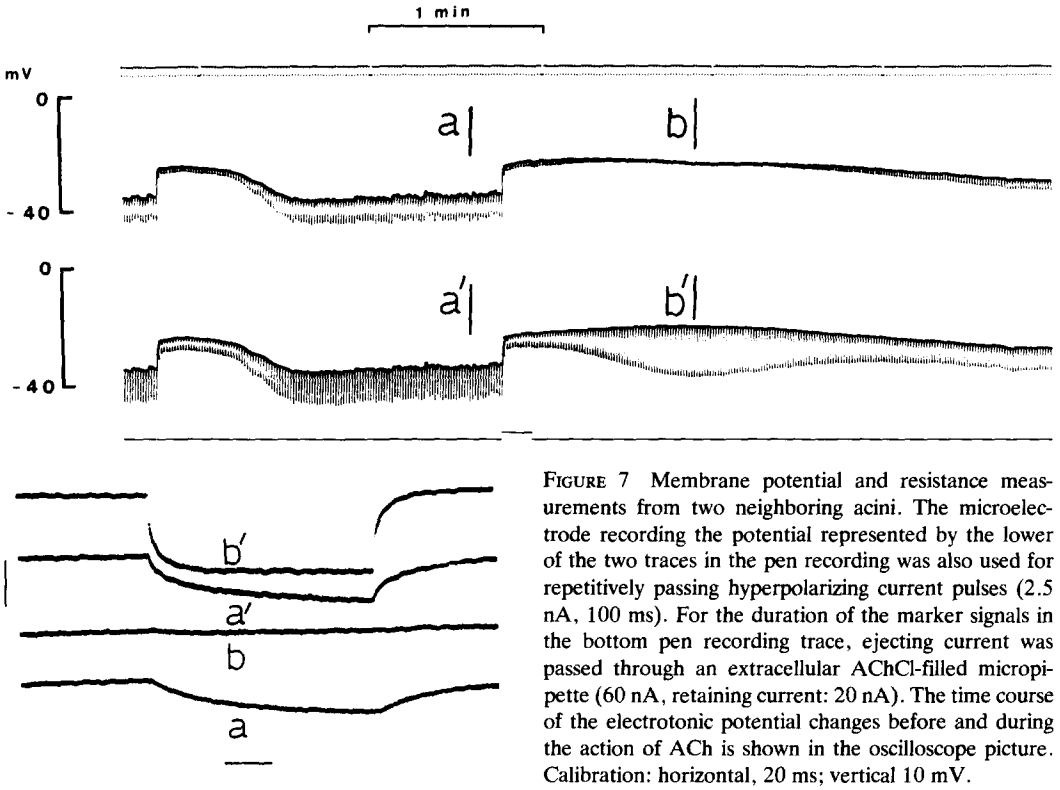

FIGURE 7 Membrane potential and resistance measurements from two neighboring acini. The microelectrode recording the potential represented by the lower of the two traces in the pen recording was also used for repetitively passing hyperpolarizing current pulses (2.5 nA, 100 ms). For the duration of the marker signals in the bottom pen recording trace, ejecting current was passed through an extracellular AChCl-filled micropipette (60 nA, retaining current: 20 nA). The time course of the electrotonic potential changes before and during the action of ACh is shown in the oscilloscope picture. Calibration: horizontal, 20 ms; vertical 10 mV.

four experiments in which ACh was used in a concentration of 10^{-5} M (the concentration needed to evoke maximal depolarization and amylase secretion in mouse fragment preparations [26]), clear decoupling responses were obtained. It was, however, possible to obtain increases in junctional resistance with even smaller ACh concentrations (1 μM) (eight experiments) (Fig. 9). In the experiment shown in Fig. 9, the coupling coefficient was 1.0 before the ACh stimulation. ~6 min after

start of superfusion with ACh (1 μM), the coupling coefficient had been reduced to 0.4. A few minutes after cessation of stimulation, the coupling coefficient had returned to the prestimulation value of 1.0. The time constant (RC) of the current pulse-induced electrotonic potential change in the current injection cell (V_1) was 9 ms before stimulation (Fig. 9,*a'*), 5 ms at the height of uncoupling (Fig. 9,*b'*), and again 9 ms after recovery from stimulation (Fig. 9,*c'*). Since the input resistance (R) at the height of uncoupling was the same as in the resting state, this means that stimulation with ACh (1 μM) reduced the effective surface area (capacitance) of this electrical unit to 55% of the resting value. It might be argued that the gradual rise in V_1 seen during exposure of the tissue to ACh could possibly be explained by desensitization. However, in spite of the fact that V_1 rose to the prestimulation value at the time of maximal uncoupling, the membrane potential was only −21 mV, while before stimulation it was −30 mV. More importantly, the membrane repolarized from −21 to −27 mV after removal of ACh. It should also be noted that V_1 decreased markedly after discontinuation of stimulation (recoupling) before again increasing (increase in surface cell membrane resistance). These results clearly cannot be explained by desensitization. In fact, even much more prolonged cholinergic stimulation does not cause desensitization of pancreatic acinar cells (4).

Peptide-Evoked Reduction in Pancreatic Acinar Cell-to-Cell Coupling

Apart from excitation of cholinergic receptors, excitation of two peptide hormone receptor sites (cholecystokinin [CCK] and bombesin) results in electrophysiological changes in pancreatic acini (21). The smallest concentration of the CCK

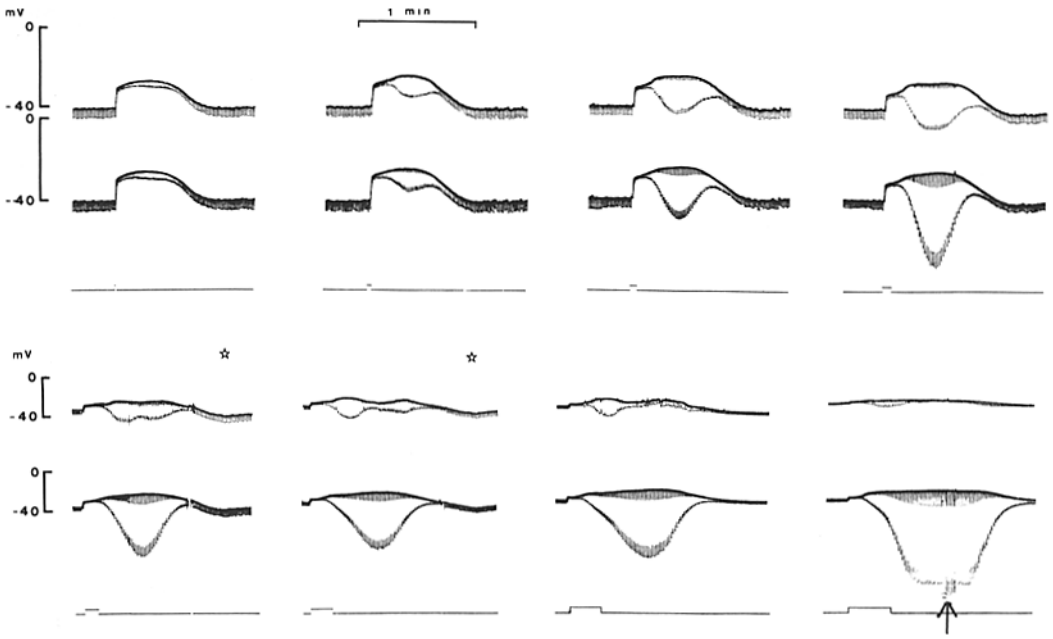

FIGURE 8 Membrane potential and resistance measurements from two cells within one acinus. The panels are consecutive tracings obtained from the same cell pair at 5-min intervals. Intracellular current pulses (2 nA, 100 ms) injected through electrode recording the membrane potential represented by the lower traces. For the duration of the marker signals in the bottom traces, ejecting current pulses (60 nA [for the first 5 panels], 90 nA [for the next two panels], and 100 nA [last panel]) were applied to ACh pipette. Note that voltage calibration in the lower part of the figure is different from that in the upper part (except for the two short sections marked with stars). In the last panel (lower, right), the pen recorder was saturated so that the size of the electrotonic potential changes in the current injection cell are shown too small except in the middle (arrow) where the correct size is shown.

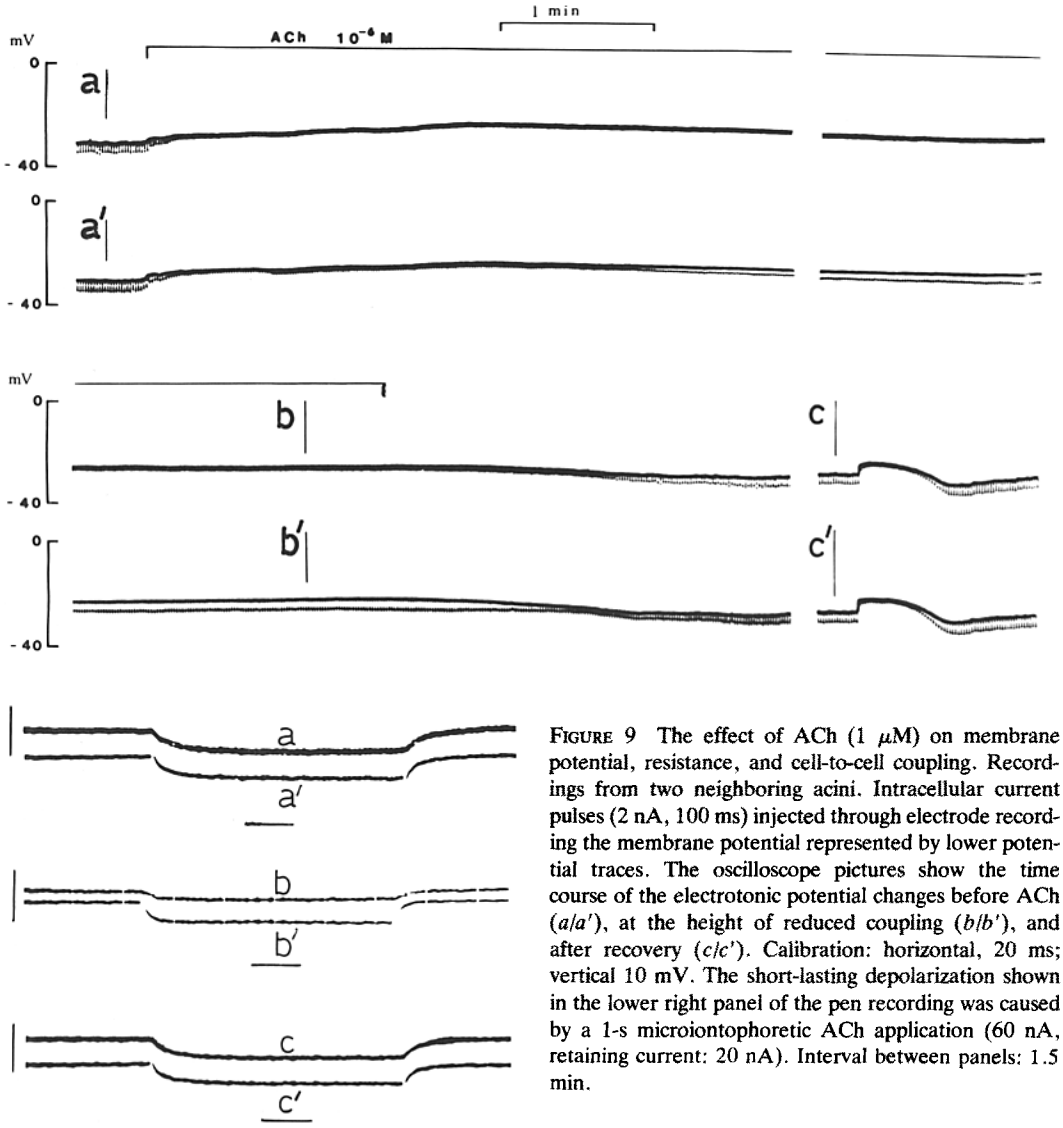

FIGURE 9 The effect of ACh (1 μM) on membrane potential, resistance, and cell-to-cell coupling. Recordings from two neighboring acini. Intracellular current pulses (2 nA, 100 ms) injected through electrode recording the membrane potential represented by lower potential traces. The oscilloscope pictures show the time course of the electrotonic potential changes before ACh (*a*/*a'*), at the height of reduced coupling (*b*/*b'*), and after recovery (*c*/*c'*). Calibration: horizontal, 20 ms; vertical 10 mV. The short-lasting depolarization shown in the lower right panel of the pen recording was caused by a 1-s microiontophoretic ACh application (60 nA, retaining current: 20 nA). Interval between panels: 1.5 min.

analogue caerulein needed to evoke maximal amylase secretion from pancreatic fragments is 1 nM while 50% stimulation is achieved at a concentration of 0.12 nM (5). Fig. 10 shows that caerulein in a concentration of 0.1 nM evoked a decrease in coupling coefficient from 1.0 to 0.4. The gradual increase in V_1 not associated with repolarization together with the concomitant reduction in coupling coefficient clearly indicates that caerulein has increased the junctional resistance. Such effects of caerulein were observed in five experiments.

The tetradecapeptide bombesin evokes maximal enzyme secretion at a concentration of 10 nM and half-maximal secretion at 0.3 nM (5, 21). In four experiments, bombesin (3 nM) evoked uncoupling responses similar to those obtained with caerulein.

ACh-Evoked Uncoupling of Lacrimal Acinar Cells

Lacrimal acinar cells within a restricted domain are electrically coupled (20). The physiological stimulants are ACh and epinephrine, and both agents cause membrane hyperpolarization associ-

ated with a marked increase in surface cell membrane conductance (20). In the pancreas, all the stimulants that evoke electrical uncoupling also cause depolarization. The lacrimal gland therefore seemed a suitable tissue in which to study whether depolarization was necessary to cause uncoupling. Fig. 11 shows the result from an experiment on a closely coupled, lacrimal acinar cell pair. A 10-s ACh pulse caused a very large increase in surface cell membrane conductance accompanied by a modest hyperpolarization. A secondary hyperpolarization occurred at a time when the conductance was beginning to return to its prestimulation level. No change in coupling coefficient was observed. More prolonged stimulation, however, led to a gradual increase in the size of the electrotonic potential changes (V_1) in the cell of current injection, clearly implicating an increase in junctional resistance. The electrical time constant was very short at the time of uncoupling, but returned to its normal value following cessation of stimulation. The uncoupling effect of ACh was fully reversible (Fig. 11). Four traces of the type shown in Fig. 11 were obtained.

DISCUSSION

The thinness of the mouse pancreas makes it an ideal preparation for direct microscopical examination of the functioning gland cells (Figs. 1 and 2). This property has been exploited in the present work on the acinar communication network by examining the spatial voltage distribution in the tissue because of a current point source placed in one cell.

Previous work has shown that neighboring cells in the pancreas are electrically coupled, and it has been suggested, on the basis of indirect evidence similar to that obtained from cockroach salivary glands (13), that the electrical unit is one acinus (31). The hypothesis that cells close together within one acinus are fully coupled has been verified (Fig. 3). It is remarkable that the coupling coefficient in such cases is always very close to 1. In the mouse liver, the coupling coefficient for neighboring cells is ~0.1 (15). This difference can be explained by the experimental observation that, in the liver, current spreads almost infinitely in all three dimensions (15, 28) whereas, in the pancreas, current spread is restricted to certain units (Figs. 3, 5, and 6). In the liver, the magnitude of electrotonic potentials as a function of distance from the current source can be precisely predicted using three-dimensional cable analysis (15). This is clearly impossible in the case of the exocrine pancreas (Fig. 6). However, even in systems limited to a small number of coupled cells, coupling coefficients are not necessarily so high as those found within pancreatic acini (12, 17, 36). The junctional resistance between acinar cells within one acinus must therefore be very low. The

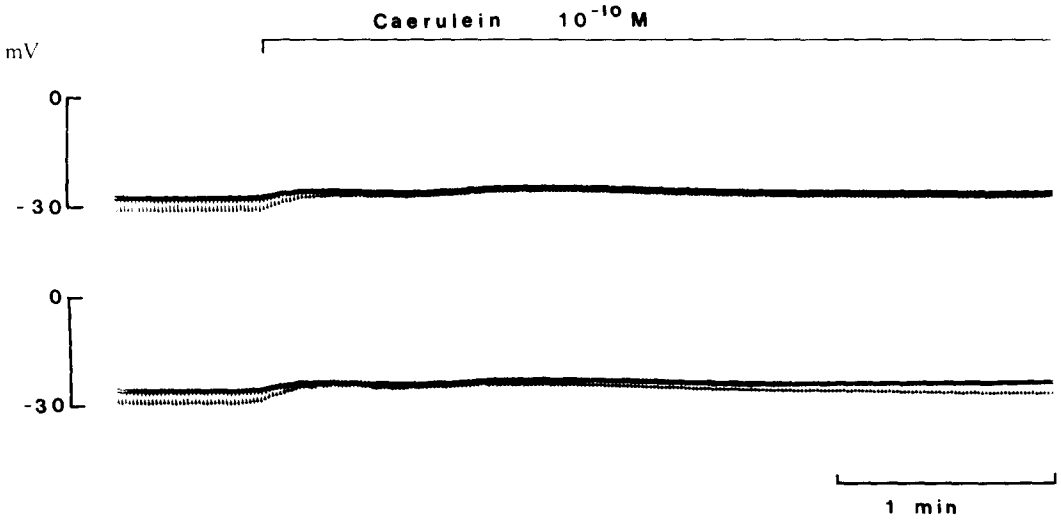

FIGURE 10 The effect of caerulein (0.1 nM) on membrane potential, resistance, and cell-to-cell coupling. Current pulses (2 nA, 100 ms) injected through electrode recording lower potential trace. Note gradual increase in size of electrotonic potential changes in current-injection cell (V_1).

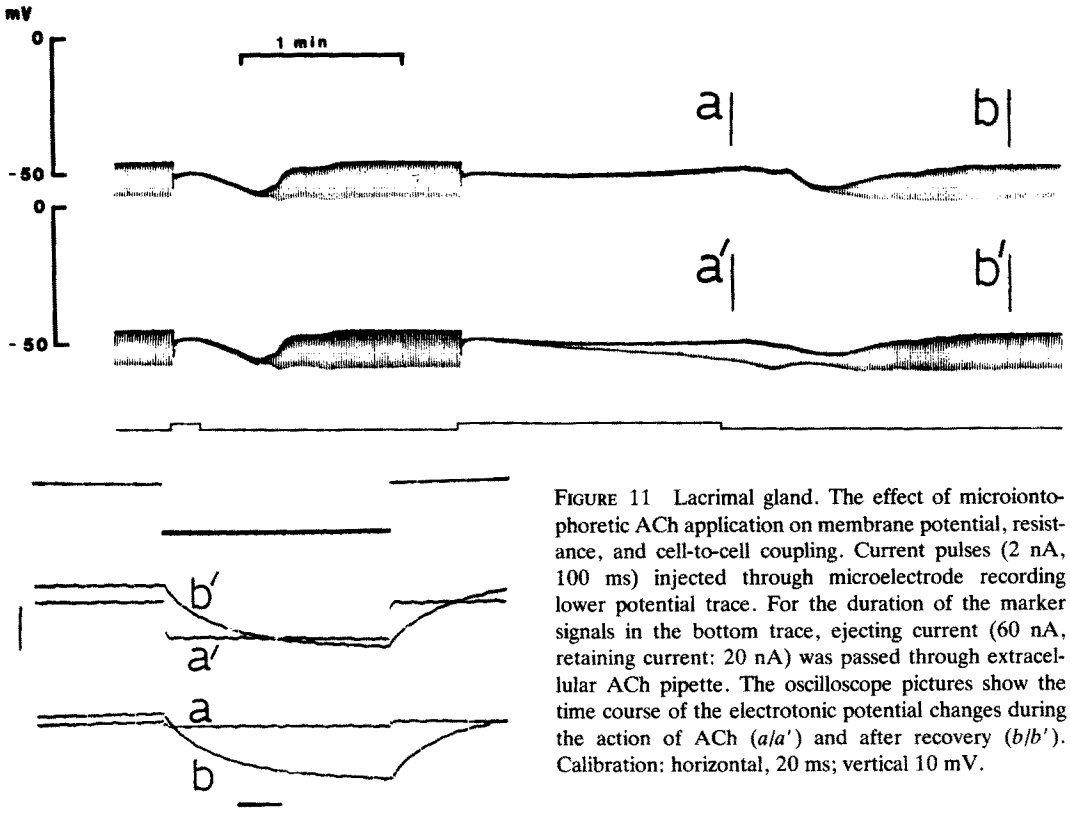

FIGURE 11 Lacrimal gland. The effect of microiontophoretic ACh application on membrane potential, resistance, and cell-to-cell coupling. Current pulses (2 nA, 100 ms) injected through microelectrode recording lower potential trace. For the duration of the marker signals in the bottom trace, ejecting current (60 nA, retaining current: 20 nA) was passed through extracellular ACh pipette. The oscilloscope pictures show the time course of the electrotonic potential changes during the action of ACh (*a*/*a'*) and after recovery (*b*/*b'*). Calibration: horizontal, 20 ms; vertical 10 mV.

extensive gap junctions demonstrated in pancreatic acini (7) are no doubt responsible for the tight electrical coupling.

The hypothesis that cells from different acini are not electrically coupled (31) may not be correct. Although it is difficult to outline exactly the borders of individual acini, coupling has in many cases been observed between what appears to be different acini (Fig. 3). Still, it cannot be rigorously excluded that what in the microscope appears as two neighboring acini could be two parts of the same acinus. It seems likely, however, that small groups of neighboring acini are the electrical units. Fig. 6 gives the distances over which coupling has been observed. These values, of course, represent the linear distances between the microelectrode tips, but the actual distance that the current must traverse, by available cellular pathways, is unknown. Neighboring acini might be electrically linked via the ducts, but we have no direct information on the possible coupling of duct and acinar cells. In fact, duct cells are not well visualized in this preparation (Fig. 1). The notion of separate electrical units in this tissue may represent an oversimplification. However, if duct and acinar cells are coupled, it seems likely that the junctional conductance between duct and acinar cells and/or between neighboring duct cells is markedly lower than between neighboring acinar cells. This, together with a long actual pathway for the current to traverse, might then explain the observed restricted communication in this tissue (Fig. 5).

The data summarized in Fig. 6 can be used to provide an estimate of the number of cells present in one electrical unit. Inspection of Fig. 6 shows that, above distances of 75 μm, very few cell pairs had high coupling coefficients (>0.5) whereas, below distances of 75 μm, almost all coupled cell pairs had high coupling coefficients. Coupling coefficients close to 1 were, however, occasionally observed over distances of up to 110 μm. As a simplifying approximation, it may therefore be assumed that the electrical unit is a sphere with a diameter of 110 μm. The volume of such a sphere would be 690,000 μm^3. According to the stereo-

logical analysis of Bolender (2) on the guinea pig pancreas, which may provide a reasonable approximation for the mouse pancreas, acinar cells take up 83% of the pancreatic volume. Since the average volume of an acinar cell is 1,060 μm^3 (2), there may be ~500 cells per electrical unit in the pancreatic acinar tissue. If one makes a further simplifying approximation and assumes that current spreads uniformly over the entire electrical unit, then, for a mean acinar cell surface membrane area of 582 μm^2 (2), a specific cell membrane resistance of 14 $k\Omega\ cm^2$ is obtained. In the mouse liver, a value of 5.1 $k\Omega\ cm^2$ has been calculated (15). In the pancreas and the liver, it is not possible to distinguish electrically between the luminal and basolateral membranes, but in the pancreas the luminal membrane area contributes <5% to the total surface membrane area (2). In the *Necturus* gallbladder and urinary bladder, it has been possible to calculate specific membrane resistances for basal and luminal membranes. In the gallbladder, values of 4.5 $k\Omega \cdot cm^2$ (basal) and 2.9 $k\Omega \cdot cm^2$ (luminal) were arrived at, whereas, in the urinary bladder, values of 1–7 $k\Omega\ cm^2$ (basal) and 9–65 $k\Omega \cdot cm^2$ (luminal) were obtained (8, 9). Combining data on the electrical time constant (RC) and the input resistance (R), the input capacitance (C) can be calculated. For R = 4.5 $M\Omega$ and RC = 15 ms, C = 3.3 nF. The surface cell membrane area of the electrical unit (500 cells) is again assumed to be $3.1 \times 10^5\ \mu m^2$. The specific membrane capacitance is therefore 1.1 $\mu F/cm^2$. This agrees well with the established capacitance value for biological membranes of 1 $\mu F/cm^2$ (6, 24).

The results presented here show that stimulants of pancreatic acinar enzyme secretion such as ACh, caerulein, and bombesin evoke increases in acinar junctional resistance even at concentrations smaller than those required for evoking maximal enzyme secretion, maximal changes in cellular Ca^{2+} metabolism, maximal membrane depolarization, or maximal cyclic nucleotide accumulation (3, 5, 10, 21, 26, 35). At higher concentrations, ACh can evoke almost total uncoupling of neighboring acinar cells (Fig. 8). All uncoupling effects are quickly and fully reversible. The stimulant-evoked electrical uncoupling between exocrine acinar cells does not depend on membrane depolarization, although in the pancreas the two processes occur together, since in the lacrimal gland ACh evokes hyperpolarization and uncoupling (Fig. 11).

In the case of the most marked ACh-evoked uncoupling so far obtained, shown in Fig. 8 (lower right panel), there was a 65-fold increase in input resistance (V_1). If one assumes that before administering the ACh pulse the acinar unit was fully coupled and that at the height of uncoupling all cells in the unit were isolated, it appears that the acinar unit from which the recording was made comprised at least 65 cells. This figure represents, of course, a lower estimate since the surface cell membrane resistance may well have decreased further during the period of uncoupling. Furthermore, we do not know whether an even larger ACh dose could have caused a further increase in input resistance. On the basis of the estimate that there are ~500 cells per electrical unit in the pancreatic acinar tissue, it would seem that, even in the experiment represented by Fig. 8, total uncoupling of neighboring cells was not quite achieved.

Socolar (38) has pointed out that in systems with high junctional conductances, consisting of relatively small units (the exocrine glands and the thyroid gland obviously belong to this group [16, 18, 19, 20, 22, 31], the coupling coefficient is very insensitive to junctional conductance changes. In cases of high junctional to nonjunctional membrane conductance ratios, there can be an almost 100-fold decrease in junctional conductance without any noticeable change in coupling coefficient. If the surface cell membrane conductance is increased markedly, changes in junctional conductance will have much more impact on the coupling coefficient (38). This relationship is probably partly responsible for the results shown in Fig. 8 where large ACh-evoked changes in coupling coefficient are seen only at very high surface cell membrane conductances.

The results presented here clearly show that all physiological stimulants of acinar enzyme secretion evoke two distinct membrane effects, one localized to the surface cell membrane consisting of an increase in conductance, the other to the junctional membranes consisting of a decrease in conductance. Although the surface cell membrane conductance change always precedes the uncoupling event in our records, this does not necessarily imply that the junctional membrane conductance change occurs after the surface membrane change, since the coupling coefficient is a function of both junctional and nonjunctional membrane conductance (38).

While the stimulant-evoked increase in surface

cell membrane conductance may be of importance for the salt and fluid secretion accompanying the enzyme secretion (18, 32), the importance of the decrease in junctional conductance is far from obvious. It is known that not only ions but also molecules up to a molecular weight of 1,200 can pass through intercellular junctions (37). Amongst the important molecules that can be transported from cell to cell are nucleotides and cyclic nucleotides (23, 29, 39). In *Chironomus* salivary glands, it is known that procedures that increase the junctional electrical resistance severely restrict the passage of molecules normally transported through the intercellular channels (34). The increase in junctional resistance occurring during stimulation in exocrine acini is therefore probably associated with a marked decrease in the flux of a variety of substances between neighboring cells.

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