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AGONIST-INDUCED CHANGES IN CELL MEMBRANE CAPACITANCE AND CONDUCTANCE IN DIALYSED PANCREATIC ACINAR CELLS OF RATS

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SUMMARY

1. Single acinar cells enzymatically isolated from the rat pancreas were subjected to tight-seal whole-cell recordings. Changes in cell membrane capacitance and conductance were simultaneously recorded using a phase-sensitive detection method.

2. Acetylcholine (ACh, 0·05–0·5 μM) and cholecystokinin octapeptide (CCK-8, 10–50 pM) concomitantly induced transient increases in cell membrane current, capacitance and conductance only when cytosolic Ca^{2+} was weakly chelated by EGTA (70 μM). These responses were prolonged when the cells were dialysed with a solution containing GTPγS (a stable analogue of GTP, 50–100 μM), whereas they were inhibited by dialysing with that containing GDPβS (a stable analogue of GDP). These results suggest that a type of guanine-nucleotide-binding protein (G-protein) could be involved in ACh- or CCK-receptor signalling.

3. The ACh- or CCK-induced responses (with or without GTPγS in the cytosol) were all abolished when a high dose of EGTA (1–2 mM) was injected into the acinar cells. In addition, A23187, a calcium ionophore, induced sustained responses when the cytosolic Ca^{2+} was weakly buffered by 70 μM-EGTA. These results suggest that the secretagogues regulate the changes in cell membrane capacitance and conductance via an increase and decrease of cytosolic Ca^{2+} concentration.

4. Oscillatory changes in cell membrane conductance and capacitance were consistently observed even without applying secretagogues when the cells were dialysed with a solution containing GTPγS (50–100 μM) and cytosolic free Ca^{2+} ions weakly buffered at about 10^{-6} M with a low dose of EGTA and $CaCl_2$.

5. The peak amplitude of changes in cell membrane capacitance induced by ACh or CCK-8, with or without GTPγS in the cytosol, varied between 200 and 1000 fF, thereby suggesting that 20–100 zymogen granules can fuse with the luminal cell membrane in response to these agonists in rat exocrine pancreatic acinar cells.

INTRODUCTION

Pancreatic acinar cells secrete a variety of digestive enzymes by exocytosis (Meldolesi, DeCamilli & Peluchetti, 1974; Palade, 1975; Case, 1979) and NaCl-rich

fluid by transporting electrolytes and water from the basolateral to the luminal side (Dockray, 1972; Petersen, Maruyama, Graf, Laugier, Nishiyama & Pearson, 1981; Petersen, 1987). Each of these secretory processes is under the control of gut hormones and neurotransmitters via activation of receptors (Gardner & Jensen, 1987), generating cellular messengers (Hootman & Williams, 1987). Although the secretion occurs in the luminal cell membrane in polarized exocrine cells, the basolateral cell membrane is responsible for triggering the chain of events of receptor signalling and for uptake of the substrates: electrolytes via the ion channels (Petersen, 1987) and amino acids via the transporter (Jauch, Petersen & Lauger, 1986; Norman & Mann, 1987). The process for the transduction of signals in 'calcium-mobilizing' receptors in the acinar cells of the exocrine pancreas has been suggested to involve hydrolysis of phosphoinositides (Mitchell, 1975) by phospholipase C under the regulation of a type of guanine-nucleotide-binding protein (G-protein; Merrit, Taylor, Rubin & Putney, 1986). Increased inositol trisphosphate (IP_3; Berridge & Irvine, 1984), one of the products of this hydrolysis, may initiate the release of Ca^{2+} from the IP_3-sensitive storage sites, probably located in the endoplasmic reticulum (Streb, Irvine, Berridge & Schulz, 1984; Streb, Bayerdorffer, Haase, Irvine & Schulz, 1984). The resulting rise of cytosolic Ca^{2+} may influence fluid secretion through activation of ion channels (Maruyama & Petersen, 1982*b*) and enzyme secretion through increased binding of zymogen granules to the luminal cell membrane (Miltinovic, Argent, Schulz & Sachs, 1977).

Both the activity of the ion channels and the process of the insertion–retrieval cycle of vesicular membranes (exo- and endocytosis) could be electrically monitored since they should be reflected by changes in cell conductance (or current) and capacitance. The whole-cell patch-clamp technique (Marty & Neher, 1983) in combination with the phase-sensitive detection method (Neher & Marty, 1982) make it possible to measure the changes in a single secretory cell whose cytosolic environment is chemically defined by cell dialysis (Neher & Marty, 1982; Clapham & Neher, 1984; Fernandez, Neher & Gomperts, 1984; Maruyama, 1986; Almers & Neher, 1987; Breckenridge & Almers, 1987). The present study aims to demonstrate how natural secretagogues act on both cell membrane conductance and capacitance (ionic transport and exocytotic secretion) when various guanine nucleotides, which are expected to modulate G-proteins, are intracellularly applied through patch-pipettes in dialysed single acinar cells of the rat exocrine pancreas.

METHODS

Male Sprague–Dawley rats (150–200 g) were stunned and sacrificed by cervical dislocation. Fragments (100 mg) of pancreatic glands were excised and serially treated with enzymes at 37 °C. Single acinar cells were obtained as described previously (Amsterdam & Jamieson, 1974) with the following modifications: pancreatic tissue was digested for 10 min in a collagenase (200 U/ml)-containing Krebs solution, minced, followed by a second digestion in a trypsin (1·5 mg/ml)-containing solution (no added calcium with 0·2 mM-EGTA) for 5 min, and finally by a third digestion in a collagenase-containing solution for 3 min. At the end of this period there were isolated single cells as well as clusters containing up to ten cells. The cells were rinsed with an enzyme-free physiological solution and stored at 4 °C. Electrical recordings were carried out within 60 min after the dispersion, using these freshly isolated single acinar cells.

The standard bathing solution contained (in mM): NaCl, 140; KCl, 4·7; $MgCl_2$, 1·13; $CaCl_2$, 2·5; Na–HEPES buffer, 5 (pH 7·2). Unless otherwise indicated, the pipette solution contained (in mM): potassium glutamate, 145; $MgCl_2$, 6·8; EGTA, 0·07; ATP, 0·5; Na–HEPES buffer, 5 (pH 7·2).

The standard whole-cell voltage-clamp technique (Marty & Neher, 1983) was used on the acinar cells whose input capacitance ranged from 7 to 9 pF ($7·2 \pm 1·5$ pF, mean $\pm$ S.D., $n = 40$). All the experiments were performed at room temperature (20–22 °C). The set-up for the electrical recordings and the fabrication method of patch pipettes were similar to those described previously (Maruyama, 1987). The resistance of the pipettes, filled with the pipette solution, varied between 5 and 8 MΩ, and the series resistance of the whole-cell recording between 12 and 23 MΩ.

Simultaneous measurement of changes in cell capacitance (C) and conductance (G) in the whole-cell recording mode were carried out using a two-phase lock-in amplifier (NI-574, NF-Instruments, Yokohama, Japan) as originally described by Neher & Marty (1982). The capacitative transient under the whole-cell configuration was neutralized by simultaneous adjustment of C_{fast}, C_{slow} and G_{series} trimmers of the amplifier (L/M EPC-7, List, Darmstadt, FRG) using square pulses. A sine-wave voltage of 20 mV peak-to-peak (800 Hz) was then added to the holding potential (−30 or 0 mV), and the resultant current was fed into the lock-in amplifier. In order to determine the correct phase setting (to avoid the cross-talk between G and C), the phase offset of the lock-in amplifier was set to an optimal position at which varying the C_{slow} trimmer caused no detectable changes in the output for G. Comparing the C calibration given by the C_{slow} trimmer during the experiment with that calculated from values of experimental parameters (the amplitude and frequency of command sine-wave voltage, the sensitivity of the lock-in amplifier, and the amplification of the recording system), it was found that the difference between these two calibrations was less than 10% (the former being smaller than the latter). This implies that the experimental error associated with the C measurement was less than 10%. The calibrations for G in the figures and text were calculated by assuming the optimal phase setting was achieved. The experimental error associated with the G measurement could be estimated from the type of experiment shown in Fig. 5 (simultaneous measurement of G and current change; see Results), and was less than 5%.

For stimulating the cell, one of the secretagogues (acetylcholine, cholecystokinin and A23187), dissolved in a standard solution, was delivered from a nearby pipette (tip diameter 5–10 μm) by weak continuous positive pressure. Each stimulation was achieved by bringing the pipette within 10 μm of the cell (see Fig. 7, inset). This method was found to minimize artifactual changes in the capacitance of pipette–cell assembly due to changes in the depth of the bathing solution with application of secretagogues.

Materials

Salts, EGTA, HEPES, ATP (sodium salt), trypsin (type XI), and ACh (acetylcholine chloride) were obtained from Sigma Chemical (St Louis); CCK-8 (cholecystokinin octapeptide) from Peninsula Lab. Inc. (Belmont, CA, U.S.A.); GTP (guanosine triphosphate), GTPγS (guanosine 5-(γ-thio)triphosphate tetralithium salt), GDPβS (guanosine 5-(β-thio)diphosphate trilithium salt) and A23187 from Boehringer (Mannheim, F.R.G.); collagenase (crude type) from Wako Chemical (Tokyo). A23187 was dissolved in ethanol, and diluted in the experimental solution to a final concentration in which the ethanol was less than 0·01%.

RESULTS

Agonist-evoked currents in dialysed single acinar cells

Figure 1 shows typical examples of secretagogue-evoked currents recorded with the whole-cell configuration in single acinar cells. The pipette solution contained 70 μM-EGTA (free Ca^{2+} ion concentration should be on the order of 10^{-8} M). The cells were voltage-clamped at 40 mV where the current was expected to be outward given the ionic gradients across the cell membrane, since either calcium-activated non-selective cation or Cl^- channels are present in the cell membrane (Petersen *et al.* 1981; Maruyama & Petersen, 1982*a*; 1984; Petersen & Maruyama, 1983). A calcium ionophore, A23187 (0·1 μM), caused a sustained outward current which persisted as

long as the cell was exposed to the solution containing this drug (Fig. 1*A*), indicating that the outward current was induced by an increase in cytosolic Ca^{2+}.

In contrast, upon stimulation by acetylcholine (ACh; 50–500 nM), the current developed to a maximum in 10 s and subsequently decayed to the original level during the stimulation. Upon repeated stimulation within several minutes, the

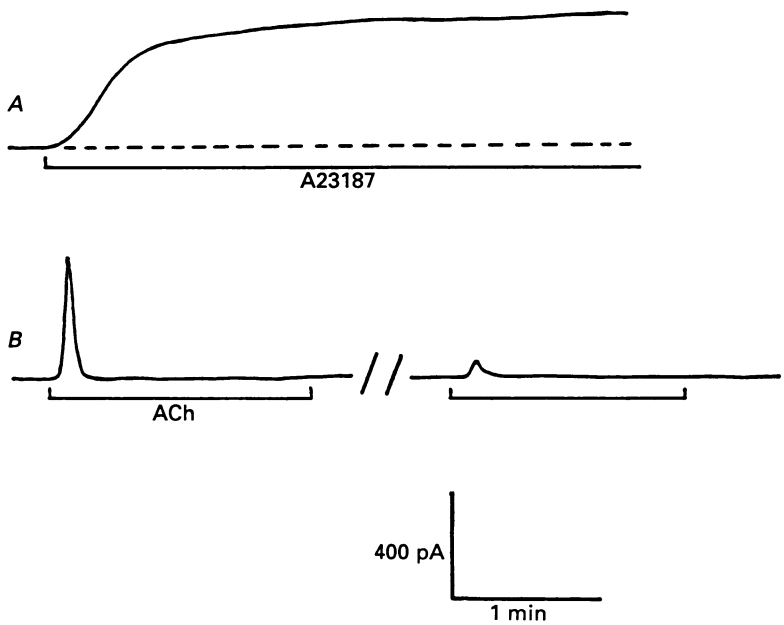

Fig. 1. Agonist-induced outward current in dialysed single acinar cells of rat exocrine pancreas. Cells were clamped at 40 mV. The upward direction represents outward current. *A*, the outward current induced by 0·1 μM-A23187. *B*, the outward current induced by 0·1 μM-acetylcholine, in which the interval between the first and second stimulation was 3 min.

amplitude of current responses to ACh was diminished (Fig. 1*B*). The threshold ACh concentration needed to observe the response was about 30 nM, at which some cells (6/10) showed a nearly full response but some (4/10) showed no response. The reason for this all-or-none type behaviour is unknown. ACh at concentrations below 10 nM never induced the response whereas 50 nM or more always induced the full response. Similar transient responses were also observed by stimulation with cholecystokinin octapeptide (CCK-8; 10–50 pM; Fig. 3*B*). The threshold CCK-8 concentration was 7 pM at which five out of eight cells showed no response but the other three cells displayed a near-maximal response. CCK-8 at concentrations less than 3 pM never induced the response whereas 10 pM or more always induced the full response. These features were qualitatively similar to those observed in the ACh stimulation.

All of these responses evoked by ACh, CCK-8 or A23187 were abolished when the cells were dialysed with a solution containing a higher dose of EGTA (1 mM). These data suggest that the stimulation with ACh and CCK-8 brought about increases in cytosolic free Ca^{2+}, but the response became desensitized after repeated stimulations.

Effects of guanine nucleotides on acetylcholine- and cholecystokinin-evoked current

Effects of various guanine nucleotides on the outward current induced by ACh (ranging from 0·2 to 500 nM) or CCK-8 (0·02–50 pM) were examined using the dialysed acinar cells. The cell membrane was clamped at 40 mV throughout the experiments (EGTA included in the pipette solution was 70 μM).

When GDPβS (guanosine 5-(β-thio)diphosphate, a non-hydrolysable GDP analogue) of 100–200 μM was applied through the patch pipette, ACh induced little or no response (Fig. 2*A*). In separate cells, current responses to ACh were not affected by intracellularly applied GTP (guanosine triphosphate, 200–500 μM; Fig. 2*B*). However, intracellular application of GTPγS (guanosine-5′-(γ-thio)triphosphate, a non-hydrolysable GTP analogue; 50–100 μM) resulted in the potentiation of the ACh-evoked response. Cytosolic GTPγS always caused a prolongation of the response with higher doses of ACh ranging from 50 to 500 nM (Fig. 2*C–F*). Typical patterns of the response included sustained (*C*), oscillatory (*D* and *E*), or transient (*F*) waveforms. The oscillatory response occasionally persisted for several minutes after the stimulation ceased (Fig. 2*E*). Moreover, cytosolic GTPγS could induce the response with lower doses of ACh, ranging from 5 to 20 nM. The response, induced by the lower doses of ACh in the presence of the cytosolic GTPγS, started with several small waves after a silent period of 5–10 s upon stimulation by ACh. The waves gradually developed to the maximum and subsequently decayed to the original level with or without oscillatory waves during stimulation. The peak size of the largest wave was comparable to that of the response evoked by the higher doses of ACh without the GTP analogue. The minimum ACh concentration needed to detect the response in the presence of GTPγS was about 1 nM (at which only small waves of 20–30 pA were observed) which was 30 times lower than the threshold ACh concentration obtained in the absence of GTPγS. ACh at concentrations less than 0·5 nM induced no response even in the presence of the cytosolic GTPγS.

Effects of the cytosolic guanine nucleotides on the current induced by CCK-8 (0·02–50 pM) were similarly examined in the dialysed acinar cells. The results were qualitatively similar to those observed in the experiments using ACh. The cytosolic GDPβS diminished the CCK-8-evoked response, the exogenous GTP caused no marked change, and the cytosolic GTPγS prolonged the current response with higher doses of CCK-8 (10–50 pM; Fig. 3*A* as an example) while it could induce the response with lower doses of CCK-8 (0·1–3 pM) at which no response was observed in the absence of the GTP analogue. The appearance of the response, induced by the lower doses of CCK-8 in the presence of the cytosolic GTPγS, was similar to that observed at lower doses of ACh. The minimum CCK-8 concentration needed to detect the response in the presence of cytosolic GTPγS was about 0·05 pM which was a dose 1/140th the threshold CCK-8 concentration in the absence of the GTP analogue. CCK-8 at concentrations less than 0·03 pM caused no response even in the presence of the cytosolic GTPγS.

All of the responses evoked by ACh or CCK-8 in the presence of the cytosolic GTPγS were abolished when the cells were dialysed with a solution containing a higher dose of EGTA (1 mM), suggesting that the effect of GTPγS is mediated by an increase in cytosolic free Ca^{2+} ions.

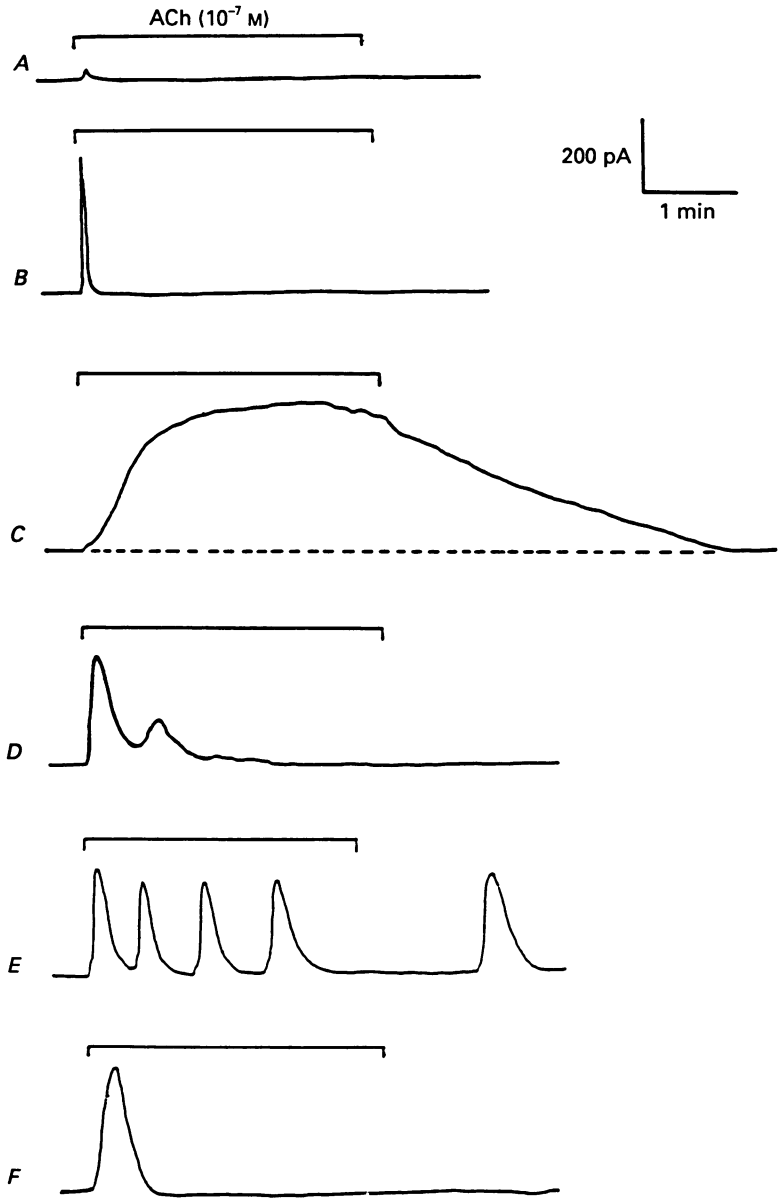

Fig. 2. Effects of guanine nucleotides on outward currents induced by a physiological dose of acetylcholine. Cells were voltage clamped at 40 mV, and stimulated by 0·1 μM-acetylcholine several minutes after equilibrium with the pipette solution containing guanosine nucleotide (*A*, GDPβS, 200 μM; *B*, GTP, 200 μM; *C–F*, GTPγS, 100 μM). Free Ca^{2+} ions in the pipette solution were loosely buffered by 70 μM-EGTA. Horizontal bars in each trace show the period of ACh stimulation. The upward direction represents outward current.

Calcium-activated currents in internally dialysed acinar cells

When the acinar cells were dialysed with the solution containing GTPγS (50–100 μM), with weak Ca^{2+}-buffering conditions (60 μM-Ca^{2+} and 77 μM-EGTA; 70 μM-$Ca^{2+}$and 77 μM-EGTA; no added Ca^{2+} and EGTA: free [Ca^{2+}] 10^{-6}–5×10^{-6} M under

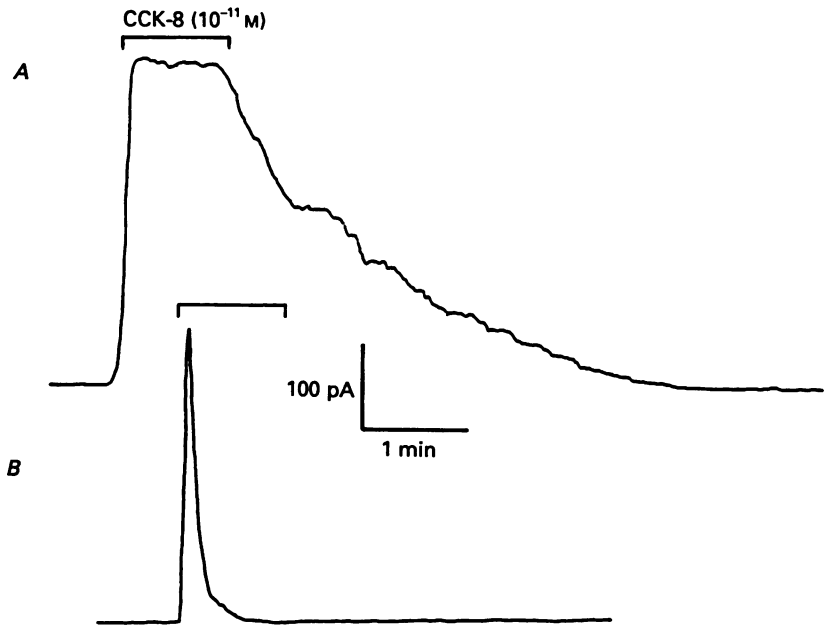

Fig. 3. Cholecystokinin-induced outward current in dialysed acinar cells. The outward current (upward deflections) induced by CCK-8 of 10 pM in the cells loaded with (*A*) or without (*B*) GTPγS of 100 μM. The holding potential was 40 mV, and free Ca^{2+} ions in the pipette solution were loosely buffered by 70 μM-EGTA.

these conditions), quasi-periodical waves of the outward current were induced without agonist (current traces in Figs 4 and 5). In contrast, when the free Ca^{2+} concentration in the pipette solution was strongly buffered at 10^{-6} to 5×10^{-6} M (0·85 or 1·0 mM-Ca^{2+} and 1·1 mM-EGTA; with or without GTPγS in the pipette solution), only a sustained increase in the outward current was observed. However, GTPγS induced no response in the cells dialysed with the solution containing EGTA of 70 μM or more but no added Ca^{2+} (pCa ~ 8).

The peak amplitudes of the oscillatory currents, evoked by the cytosolic GTPγS and weakly buffered Ca^{2+}, were plotted against voltage and compared with that obtained from the ACh-evoked current (a typical example of each *I–V* relation is shown in Fig. 4). For obtaining the *I–V* relation of peak ACh-evoked currents, GTPγS of 100 μM was included in the pipette solution, which made it possible to induce reproducible responses to brief repeated applications of ACh. In records of the ACh-evoked response similar to those shown in Fig. 4(*a–c*), it was observed that the peak response was slightly delayed at membrane potentials where the peak currents

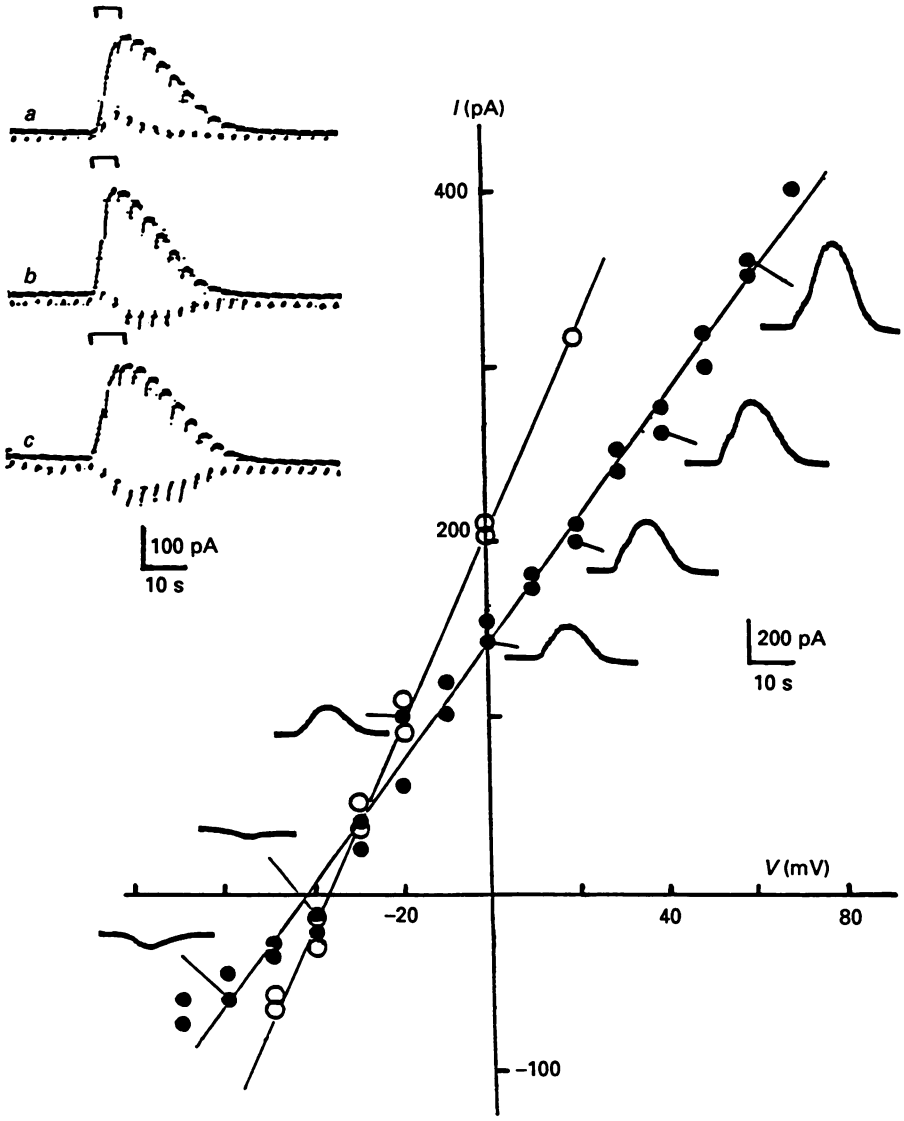

Fig. 4. A typical peak current–voltage relationship of oscillatory current waves (●) and that of acetylcholine-evoked current (○). GTPγS of 100 μM was included in the pipette solution in both cases. Oscillatory currents were induced by cytosolic dialysis with GTPγS and a loosely buffered Ca^{2+} solution (no added Ca^{2+} and EGTA). *a–c*, a typical example of ACh-evoked currents recorded at various potentials, in which voltage pulses of 500 ms in duration were repetitively applied from 0 to −30 mV (*a*), to −30 mV (*b*) and to −50 mV (*c*), and ACh was applied during the period indicated by a horizontal bar. In the current traces, the upward direction represents outward current.

were inward, and that a biphasic response was frequently present at the potential near to the reversal potential of the peak current. The properties of the peak oscillatory waves and the peak ACh-evoked currents, obtained from several different experiments in each case, were similar in conductance and reversal potential. The conductance of the oscillatory and the ACh-evoked response was linear and was

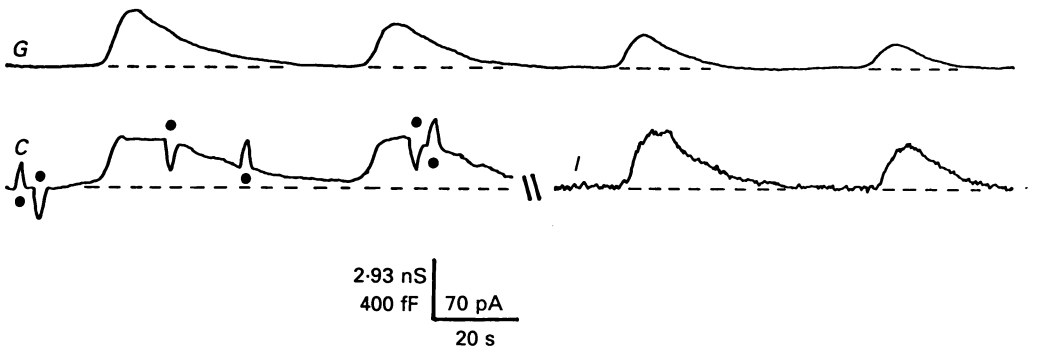

Fig. 5. Oscillatory changes in cell membrane capacitance, conductance and current induced by exogenously applied GTPγS. Single acinar cell was dialysed with the pipette solution containing no added Ca^{2+} and 100 μM-GTPγS. The holding potential was 0 mV. During the recording, the calibration signals (●) for capacitance were frequently applied to confirm the optimal phase setting. The output of cell capacitance (*C*) was switched to that of cell current (*I*) during the continuous monitoring of cell conductance (*G*). The upward direction represents increases in capacitance and conductance and outward current. The last two peaks of the cell conductance were 1·67 and 1·21 nS, whereas corresponding current peaks were 67·5 and 50·0 pA which give conductances of 1·68 and 1·24 nS, respectively, if divided by the voltage difference between the holding potential (0 mV) and zero-current potential (−40 mV).

3·4±1·8 nS (mean±S.D., $n = 5$) and 3·2±1·4 nS ($n = 5$), respectively. The zero-current potential of these responses was −39±6·3 mV ($n = 5$) and −40±7·2 mV ($n = 5$), respectively, when using the standard extracellular and pipette-filled solutions, and was −7±4 mV ($n = 4$) and −5±6 mV ($n = 3$), respectively, under symmetrical Cl^- gradients across the cell membrane (potassium glutamate in the pipette solution was replaced by an equimolar KCl). Thus, these two current responses could be due to the openings of the same ion channels. Although systematic studies of ion selectivity in the current response have not been performed, it is likely that the current response is due to the openings of both calcium-activated non-selective cation channels demonstrated by the single-channel recording (Maruyama & Petersen, 1982*a*, *b*) and calcium-activated Cl^- channels suggested by micro-electrode experiments (Iwatsuki & Petersen, 1977; Petersen & Maruyama, 1983) and whole-cell recordings (Randriamampita, Chanson & Trautmann, 1988).

Oscillatory changes in capacitance and conductance evoked by cytosolic GTPγS

Oscillatory waves induced by the cytosolic GTPγS and the weakly buffered Ca^{2+} (a mixture of 60 μM-Ca^{2+} and 77 μ-EGTA, or no added Ca^{2+} and EGTA, in the pipette solution) were studied in terms of changes in cell membrane capacitance (*C*)

and conductance (G) using the phase-sensitive detection method. These responses usually appeared several minutes after equilibrium with the pipette solution. Figure 5 shows traces for two outputs of the lock-in amplifier (for C and G) and the actual current trace (I) low-pass filtered at 80 Hz. In the C trace, the calibration signals of

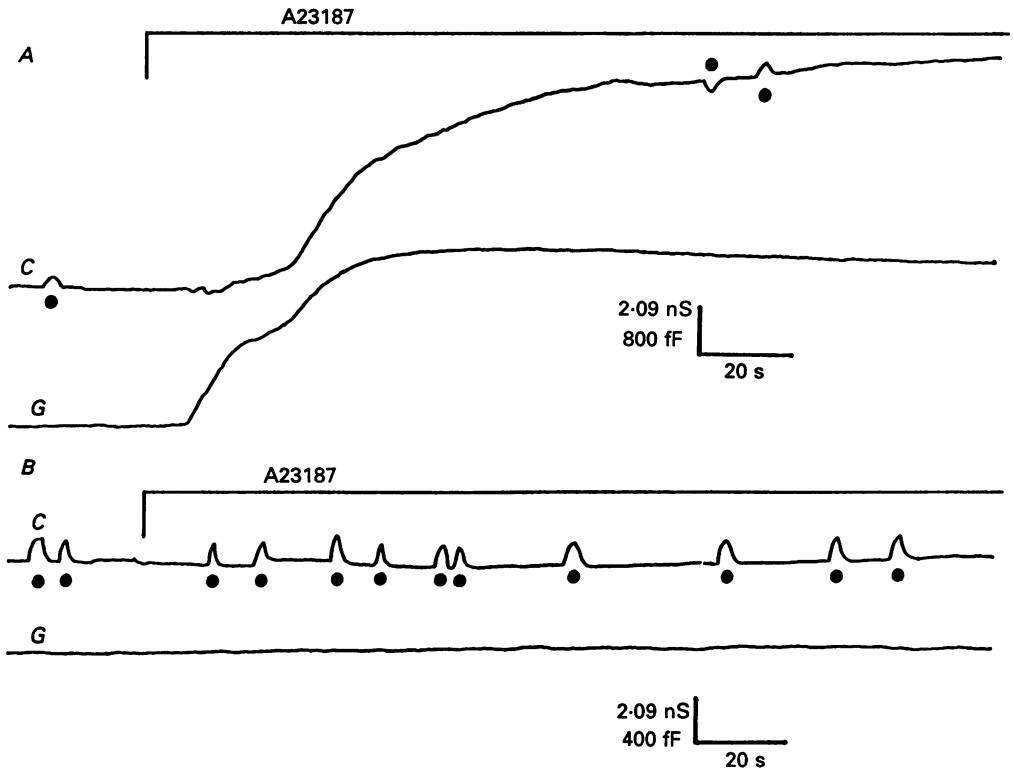

Fig. 6. A23187-induced changes in cell membrane capacitance and conductance. Cells were dialysed with the pipette solution containing EGTA of either 70 μM (A) or 2 mM (B), and stimulated by A23187 of 0·3 (A) or 1·0 μM (B). In the trace of cell capacitance (C), ● shows the calibration signals given by the capacitance neutralization circuit. The holding potential was 0 mV. The upward direction represents increases in capacitance and conductance.

200 fF given by the C-neutralizing circuit caused little changes in G, indicating that the optimal phase setting for the separation of C from G was achieved. Moreover, the changes in the current (I) were exactly tracked by that of G if the I value was converted into the conductance by calculation (the current was divided by voltage difference between the holding potential and the zero-current potential; the zero current potential was $-40 \text{ mV} \pm 7{\cdot}2 \text{ mV}$, $n = 5$), indicating that changes in G reflected the activities of calcium-activated channels and the errors associated with the measurements of C and G were very small.

Changes in cell capacitance and conductance induced by A23187

The calcium ionophore A23187 (0·1–1·0 μM) could evoke a sustained increase in either cell membrane capacitance (C) or conductance (G) when Ca^{2+} in the pipette

solution was loosely buffered by 70 μM-EGTA (Fig. 6*A*). However when tightly buffered by 2 mM-EGTA, it caused no response (Fig. 6*B*). This indicates that the cytosolic Ca^{2+} is an important factor regulating the activities of ion channels and vesicular membrane exocytosis. The rise of *C* after stimulation showed a clear delay

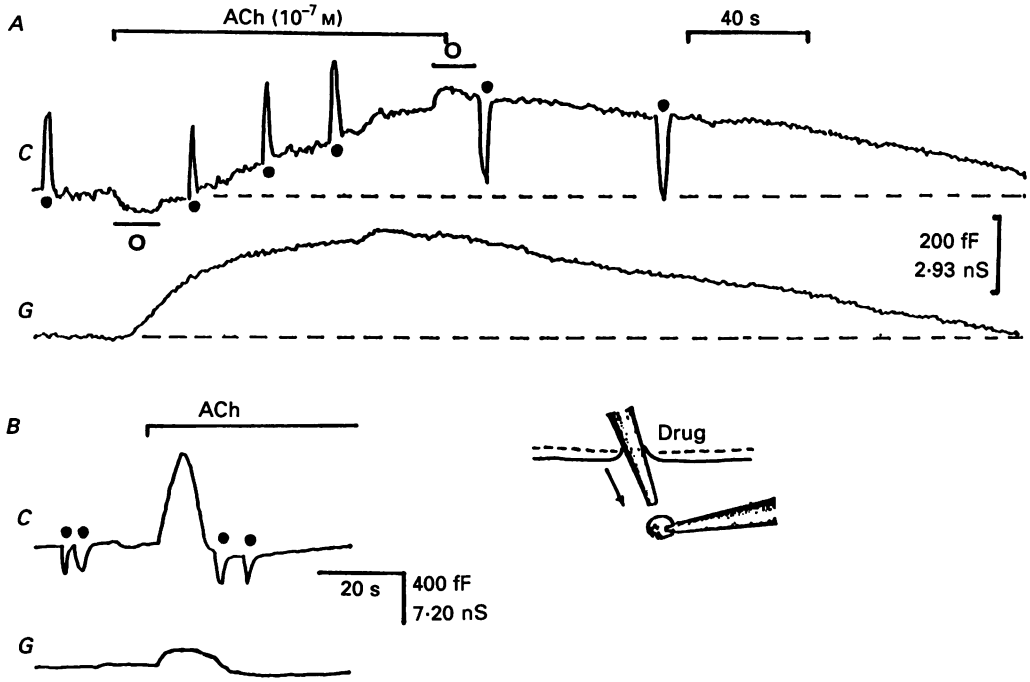

Fig. 7. Acetylcholine-induced changes in cell membrane capacitance (*C*) and conductance (*G*). Cells were dialysed with the pipette solution containing either 100 μM-GTPγS (*A*) or no guanine nucleotide (*B*). Acetylcholine (0·1 μM) was applied during the period indicated by horizontal bars. Upward and downward deflections (●) in the trace of capacitance are the calibration signals of 200 fF. The upward direction in each trace represents an increase in the response. The small changes in capacitance (○ in *A*) reflect the changes in solution surface (changes in capacitance of pipette–cell assembly) when the drug application pipette was immersed in or withdrawn from the solution. The level of the solution surface was slightly decreased due to surface tension when the drug pipette was immersed into the solution. Inset illustrates the application procedure of the drug.

relative to that of *G* (Fig. 6*A*). This delay was consistently observed in the case of the A23187 stimulation, but not by ACh (Fig. 7) or CCK-8. The changes in cell membrane capacitance induced by A23187, measured in the sustained phase, ranged from 2·5 to 4·3 pF.

Acetylcholine-induced changes in cell membrane capacitance and conductance

Upon stimulation of the acinar cells by ACh (0·08–0·5 μM), transient increases in cell membrane capacitance and conductance were observed only when Ca^{2+} in the pipette solution was weakly buffered by 70 μM-EGTA (Fig. 7*B*). These responses were prolonged, as in the case of the current measurement, when GTPγS (50–100 μM) was applied to the cells (Fig. 7*A* is a typical example). In addition, 10 nM-ACh

induced responses (in the presence of 100 μM-cytosolic GTPγS) of comparable size to those induced by 0·1 μM-ACh in the absence of the GTP analogue. Without the cytosolic GTPγS, 10 nM-ACh never induced responses. The responses were abolished when GDPβS (100–200 μM) or EGTA (more than 0·7 mM) was administered in the pipette solution. GTP caused no marked changes in the responses as in the case of the current recordings. In contrast to the effect of A23187, the time course of changes in C was similar to that of G, without showing a distinct delay (Fig. 7A). The peak increase in C induced by ACh varied between 200 and 1000 fF, which was 3–14% of the acinar cell input capacitance (7·2 $\pm$ 1·5 pF, mean $\pm$ S.D., $n = 40$).

The same experimental protocols as in the case of ACh were repeated using cholecystokinin-octapeptide (CCK-8, 10–50 pM) in the acinar cells whose cytosolic Ca^{2+} was weakly buffered by 70 μM-EGTA. Cell responses to CCK-8, when modified by guanine nucleotides, were qualitatively similar to those obtained from the experiments using ACh. The responses in I, G and C were all eliminated when the pipette solution was strongly chelated by a higher dose of EGTA (0·7 mM or more). The changes in C and G almost coincided with each other.

DISCUSSION

The basic findings of this study are that ACh and CCK-8 induced increases not only in the cell membrane capacitance but also in the cell membrane conductance, and that both responses were regulated by receptor-coupled G-proteins and mediated by intracellular Ca^{2+} ions. Cytosolic GTPγS potentiated the ACh- or CCK-8-induced responses which were either inhibited by the cytosolic GDPβS or abolished by higher doses of EGTA.

The capacitance increase induced by ACh or CCK-8 ranged from 200 to 1000 fF. Since the membrane capacitance of a zymogen granule has been estimated to be around 10 fF (Maruyama, 1986), it is likely that stimulation with a physiological dose of ACh or CCK-8 brought about insertion of 20–100 zymogen granules by exocytotic membrane fusion. The resultant increase in the surface area of the luminal cell membrane would be 20–100 μm^2 when the specific capacitance of 1 $\mu F/cm^2$ is assumed. This implies that the area of the luminal cell membrane increased by 2- to 4-fold, since that of an unstimulated pancreatic acinar cell has been reported to be 30 μm^2 (Bolendar, 1974). Similarly, it can be estimated that A23187 induced 8- to 14-fold increases in the area of the luminal cell membrane by the insertion of 250–430 zymogen granules. It has been known that cytosolic Ca^{2+} ions play a prerequisite role in the exocytotic process in the acinar cells (Hootman & Williams, 1987). In fact, relatively high doses of EGTA applied to the cytosol inhibited secretagogue-induced capacitance changes (Fig. 6B, for example), and the capacitance response was always accompanied by calcium-dependent conductance increases (Figs 5–7). A cycle of exo- and endocytosis may be a complicated process since it depends on the cytosolic Ca^{2+}, the number of granules left in the releasable pool, and the number of fusion areas available in the membrane (Knight & Baker, 1982). In the present study of ACh- or CCK-8-induced increase in C (exocytosis), one interpretation for the role of Ca^{2+} could be that the increase of cytosolic Ca^{2+} somehow facilitates the rate of granular fusion. During a continuous rise of cytosolic Ca^{2+} as in A23187 stimulation, it may be that a steady state of fusion/retrieval is attained.

ACh and CCK-8 gave rise to an increase in the membrane conductance by about 3·4 nS. The major increase in cell membrane conductance could be expected to take place in the basolateral cell membrane which has been reported to make up 95% of the total plasma membrane area (Bolendar, 1974) and to contain calcium-activated Na^+–K^+ non-selective monovalent cation channels (Maruyama & Petersen, 1982*a*; 1984). Such conductance increases in response to ACh and CCK-8 were consistently coupled with the membrane capacitance increase (Figs 5–7). A question therefore arises as to whether some ionic channels (relating to transcellular ionic transport or fluid secretion), which had existed on the zymogen granule membrane, were newly inserted into the luminal cell membrane upon exocytosis induced by these agonists. However, this possibility seems unlikely, since upon the stimulation of A23187, the capacitance response occurred after the conductance response with significant delay (Fig. 6).

In the present study, the current responses to ACh or CCK-8 were augmented by cytosolic application of GTPγS but were inhibited by GDPβS. They also depended on the cytosolic free Ca^{2+}. Merritt *et al.* (1986) have reported that the exogenous GTPγS potentiates accumulation of inositol trisphosphate (IP_3), which may cause the release of Ca^{2+} from its storage site (Streb *et al.* 1984), and that the exogenous GDPβS (300 μM) does not affect IP_3 accumulation in response to cholinergic or peptidergic agonist in permeabilized acinar cells of exocrine pancreatic gland. The effect of GTPγS (but not that of GDPβS) is consistent with their result. Moreover, Evans & Marty (1986) have demonstrated, with the whole-cell recording technique using dialysed acinar cells of lacrimal gland, that the cytosolic GTPγS greatly potentiates both the calcium-dependent cholinergic and adrenergic current responses. The effect of the cytosolic GTPγS in the present study (activation of the response in lower doses of ACh or CCK-8) is qualitatively consistent with their result. The time course of the response induced by the lower doses of ACh or CCK-8 in the presence of the cytosolic GTPγS was different from that of the higher doses of the agonists (in the lower doses the response tended to start with a lag period and small oscillatory waves, while in the higher doses it tended to develop abruptly and maximally). Underlying mechanisms of the difference are presently unknown, and this may make it difficult to estimate the GTPγS potentiation quantitatively. In addition, cytosolic GTPγS prolonged the current (conductance) response (the pattern of which was roughly divided into four types, Fig. 2*C–F*) as well as the capacitance response (Fig. 7*A*) induced by the higher doses of ACh or CCK-8. The effect of the cytosolic GTPγS both in the lower and higher doses of the agonists may be explained qualitatively by the potentiation of IP_3 production through the activation of G-proteins as suggested by Evans & Marty (1986) and Merritt *et al.* (1986).

GTP (200–500 μM) perfused into cells by a patch pipette caused no detectable effects on the responses evoked by ACh or CCK-8. The lack of the effect of GTP on the secretagogue-evoked IP_3 production has been reported in permeabilized pancreatic acinar cells (Merritt *et al.* 1986) and on the secretagogue-evoked current response in dialysed acinar cells of the lacrimal gland through a patch pipette (Evans & Marty, 1986).

The precise mechanism of the GTPγS-induced oscillatory behaviour in the cell membrane current, conductance and capacitance (Fig. 5) is presently unknown.

However, this could relate to the oscillatory changes in the concentration of cytosolic free Ca^{2+}, since this phenomenon was never observed when Ca^{2+} ions in the pipette solution were buffered by EGTA of 70 μM or more. The oscillatory changes in cytosolic free Ca^{2+} concentration somewhat resembled those reported in aequorin-loaded hepatocytes (Woods, Cuthbertson & Cobbold, 1986).

On the whole, it seems reasonable to conclude that receptor stimulation by ACh or CCK-8 results in exocytotic fusion of zymogen granules at the luminal cell membrane and activation of ionic channels in the basolateral cell membrane, and that G-proteins and calcium-mobilization are involved in both luminal exocytosis and basolateral ion channel activation, in the exocrine pancreatic acinar cells.

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