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Calcium-activated membrane depolarization via modulation of chloride efflux from parietal cells during gastrin stimulation

Yasuhiro Tsunoda and Hidemi Matsumiya

Department of Laboratory Medicine, Hokkaido University School of Medicine, Sapporo 060, Hokkaido, Japan

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In the isolated parietal cell the following observations were made: gastrin led to an increase in cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$) even in the absence of medium Ca^{2+} and to transient membrane depolarization in both the absence of the medium Ca^{2+} and the fura-2-loaded cell. The incorporated Ca^{2+} chelator BAPTA inhibited the gastrin-induced membrane depolarization. The magnitude of depolarization caused by gastrin was unchanged on removal of medium Na^+. Furosemide but not tetraethylammonium inhibited the gastrin-induced depolarization. The results suggest that the Ca^{2+} released from the store(s) induces membrane depolarization, possibly via modulation of a Cl^- efflux across the luminal surface during gastrin stimulation.

Gastrin; Ca^{2+} release; Depolarization; Cl^- efflux; (Parietal cell, Guinea pig)

1. INTRODUCTION

In mammalian gastric parietal cells the intracellular Ca^{2+}-mobilizing hormone is thought to be gastrin, since it causes an increase in aminopyrine accumulation or oxygen consumption (as an index of acid secretion) and in $[Ca^{2+}]_i$ in both the absence of medium Ca^{2+} and the presence of a Ca^{2+}-channel blocker [1–3], thus differing from histamine, which produces cyclic AMP, and from cholinergic muscarinic stimulation, which increases Ca^{2+} entry from the extracellular space [3,4]. An ATP-dependent and inositol trisphosphate-sensitive Ca^{2+} pool is located in the apical portion of the guinea pig parietal cell that comprises tubulovesicular elements and secretory canaliculi and is connected with microfilaments [5]. However, the gastrin-elicited Ca^{2+} metabolism has not yet been substantiated including the question of how Ca^{2+} released from the store(s), as an intracellular signal transducer, leads to acid secretion from the gastric parietal cell. One possibility is that Ca^{2+} regulates K^+ permeability and the subsequent activation of H^+,K^+-ATPase, implying a direct control of Ca^{2+} on K^+ channels [6]. On the other hand, the stimulation activates a Cl^- component in the secretory membrane and not in the basolateral membrane of the parietal cell via a K^+-Cl^- pathway. The Cl^- component in the

Correspondence address: Y. Tsunoda, Department of Laboratory Medicine, Hokkaido University School of Medicine, Sapporo 060, Hokkaido, Japan

Abbreviations: fura-2, 1-(2-(5′-carboxyoxazol-2′-yl)-6-aminobenzofuran-5-oxy)-2-(2′-amino-5′-methylphenoxy-ethane-*N,N,N′,N′*-tetraacetic acid; BAPTA, 1,2-bis(*o*-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetic acid; diS-C_3-(5), 3,3′-dipropylthiodicarbocyanine; Mops, 3-(*N*-morpholino)propanesulfonic acid; quin-2, 2-(2-amino-5-methylphenoxy)methyl-6-methoxy-8-aminoquinoline-*N,N,N′,N′*-tetraacetic acid

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stimulated state results from the addition of Cl^- conductance in the H^+-K^+ pump-associated membrane [7,8]. It is well known that Ca^{2+} regulates K^+ and Cl^- currents via the opening of specific channels in many exocrine cells [9,10]. Therefore, the aim of this study has been focused on defining the relationship between a functional role for Ca^{2+} released from the store(s) and the K^+ or Cl^- pathway during gastrin stimulation.

2. MATERIALS AND METHODS

2.1. *Materials*

The sources of some of the reagents mentioned below have been given previously [1,2,5,11]. diS-C_3-(5), from a stock solution of 1 mM in dimethyl sulfoxide, was obtained from Molecular Probes (USA). Aequorin was purchased from Dr Blinks, whose preparation was lyophilized from a solution containing 1 mg/ml aequorin, 5 mM Hepes and 150 mM KCl (Mayo Foundation, USA). 1 mM fura-2, 10 mM quin-2 and 50 mM BAPTA acetoxy methyl esters in dimethyl sulfoxide were obtained from Dougindo (Japan).

2.2. *Isolation of parietal cells*

Heterogenous gastric mucosal cells from guinea pig gastric mucosa (Hartley, male, 250 g) were prepared as in [2,11].

Enriched cell populations were prepared by Percoll density gradient ultracentrifugation (30000 × *g* for 15 min at 4°C) [11]. A fraction enriched in parietal cells was recovered at a density of between 1.043 and 1.050 g/ml. Chief cell contamination was less than 13.40 ± 0.32% (n = 6).

2.3. *Measurement of membrane potential*

2 μl of 1 mM diS-C_3-(5) was added to 1.7 ml Mops-Tyrode buffer (in mM: 150 NaCl, 5 KCl, 1 $MgSO_4$, 0.2 KH_2PO_4, 0.8 K_2HPO_4, 10 Mops-Na, 5 glucose, pH 7.4) with (1 mM $CaCl_2$) or without (0 mM $CaCl_2$, 1 mM EGTA) $CaCl_2$ and then 300 μl cell suspension (10^5 parietal cells in Mops-Tyrode buffer) was added to the cuvette at 37°C (final concentration of diS-C_3-(5), 1 μM). The signal was recorded at wavelengths of 620 nm for excitation and 666 nm for emission, both slit widths being 20 nm, using a Hitachi 650-60 fluorescence spectrometer (Japan). The fluorescence changes are reported as a percentage of the total full-scale fluorescence. For all measurements (including those described in section 2.4), cells were temperature equilibrated with stirring in a thermostatted cuvette at 37°C for 3 min.

2.4. *Measurements of cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$)*

2 μM fura-2 acetoxymethyl ester was added to a parietal cell suspension (10^6 cells/ml) in RPMI-1640 medium containing 10 mM Mops and 25 mM $NaHCO_3$. The cell suspension was incubated for 15 min at 37°C [13]. After loading of the dye fura-2, the cell suspension was rinsed twice and 0.25 × 10^6 cells were resuspended with 0.5 ml of low Na^+ and high K^+ Mops-Tyrode buffer (40 mM Na^+, 100 mM K^+) in the presence (1.0 mM Ca^{2+}) or absence (prepared by omitting $CaCl_2$ and adding 1 mM EGTA) of medium Ca^{2+}. Fura-2 fluorescence was detected using a CAF-100 fluorescence spectrometer (Japan Spectroscopic). The emission wavelength was set at 500 nm while the excitation wavelength was obtained from the 340/380 nm ratio [12] or maintained at 380 or 340 nm for the entire measuring period. $[Ca^{2+}]_i$ values were calculated from fura-2 ratios by the equation in [12]. The method of quin-2 (20 μM) loading was very similar to that of fura-2 [1,2]. Quin-2 fluorescence was recorded with a Hitachi 650-10LC fluorescence spectrometer. The excitation and emission wavelengths were 339 and 492 nm with 4 and 10 nm bandwidths, respectively. $[Ca^{2+}]_i$ was calculated as in [13]. Aequorin loading was performed as in [14,15]. Parietal cells were incubated (5 × 10^6 cells/150 μl) at 24°C for 6 min in Mops-Tyrode solution with 1 mM ATP and 2 mM EGTA following the addition of 10 μl aequorin (0.3 mg/ml aequorin in 5 mM Hepes + 50 mM KCl + 7 mM EGTA). Dimethyl sulfoxide was added stepwise during the incubation to a final concentration of 6%. Incubation was terminated by the addition of 900 μl Mops-Tyrode solution with 5 mM EGTA and 1 mM ATP followed by centrifugation at 12000 × *g* for 15 s. The resultant cell pellet was resuspended with 10 ml Mops-Tyrode solution. $CaCl_2$ was added to 1 ml cell suspension (5 × 10^5 cells/ml) to a final concentration of 1 mM in a cuvette and gastrin added after the signal from the Ca^{2+}-aequorin complex outside the cells had disappeared. Aequorin luminescence was determined with a platelet ionized calcium ag-

gregometer (Chrono Log, USA) with constant stirring at 37°C. $[Ca^{2+}]_i$ was calculated as in [14].

3. RESULTS

diS-C_3-(5) is a lipophilic cation whose fluorescence is quenched when it accumulates inside cells. Thus, when this dye is added to a cell suspension, the distribution between the cell-bound and free dye in the medium depends on the membrane potential. Depolarization causes a decrease in the amount of cell-associated diS-C_3-(5) and the fluorescence of diS-C_3-(5) increases when it is released from cells [16]. As shown in fig.1, addition of gastrin (10^{-8} M) to parietal cells suspended in Ca^{2+} (1 mM)-containing medium increased the fluorescence signal of diS-C_3-(5) in a transient manner which appeared to represent depolarization and subsequent re-polarization of the cell. The depolarization transient brought about by gastrin was shown even in the absence of medium Ca^{2+} (0 mM Ca^{2+}, 1 mM EGTA) and was similar in magnitude to that observed in the presence of medium Ca^{2+}. In contrast, the Ca^{2+} ionophore ionomycin-induced membrane depolarization was mainly dependent on medium Ca^{2+}. However, the small but transient depolarization (by ionomycin) was also demonstrated even in the absence of medium Ca^{2+}. Higher concentrations of ionomycin (2.5–5 μM) in the presence of medium Ca^{2+} failed to re-polarize the cells, possibly owing to no Ca^{2+} gradient being present. The acetylcholine analogue carbachol-elicited depolarization disappeared in the absence of medium Ca^{2+} which was similar to the $[Ca^{2+}]_i$ change that required medium Ca^{2+} [3].

As shown in fig.2, the increase in $[Ca^{2+}]_i$ elicited by gastrin was almost wholly due to the intracellular Ca^{2+} released from the store(s), since the omission of medium Ca^{2+} failed to decrease the signals measured with fura-2, quin-2 and aequorin. In the case of measurement with fura-2 or quin-2, the average resting level of $[Ca^{2+}]_i$ of the parietal cell was 170 and 200 nM, respectively. Gastrin

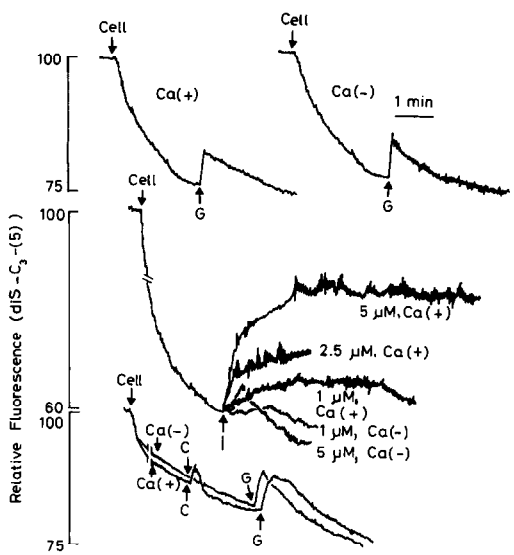

Fig.1. Membrane polarization of the parietal cell as measured by diS-C_3-(5). G, gastrin (10^{-8} M); I, ionomycin (concentrations as indicated); C, carbachol (10^{-4} M). The ligand was added 3 min after the cells. Ca(+) and Ca(−): 1 mM Ca^{2+} and 0 mM Ca^{2+} plus 1 mM EGTA in the medium, respectively (including figs 2,3). Cells added to 1 μM diS-C_3-(5) in standard medium. The trace is representative of at least five similar experiments.

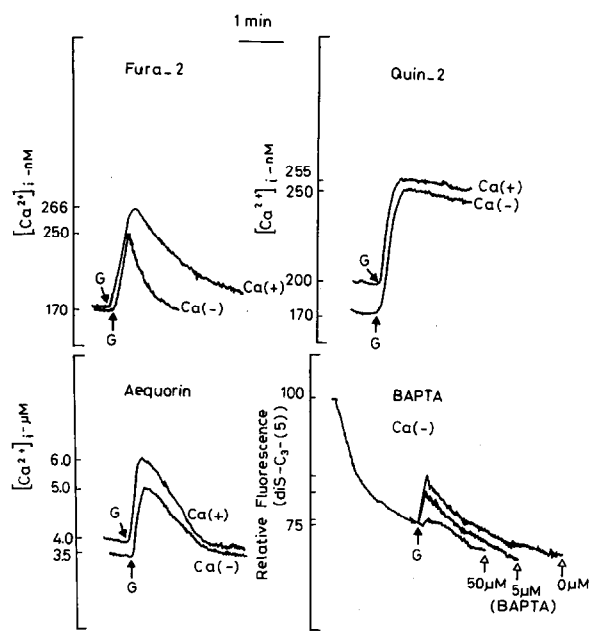

Fig.2. $[Ca^{2+}]_i$ and membrane polarization in response to gastrin. The cells were with BAPTA acetoxy methyl ester at varying concentrations for 10 min at 37°C before stimulation. G, gastrin (10^{-8} M). The trace is a representative of at least five similar experiments.

caused a rapid but transient increase in $[Ca^{2+}]_i$ even in the absence of medium Ca^{2+} (about 60–90 nM final rise). It seems unlikely that the luminescence in the resting state as measured using aequorin-loaded cells with high $[Ca^{2+}]_i$ is due to their increased permeability, since they contain micromolar $[Ca^{2+}]_i$ even in the absence of medium Ca^{2+}, thus suggesting a different Ca^{2+} gradient in the cytosol and heterogeneity of $[Ca^{2+}]_i$ [14]. Gastrin led to an increase in $[Ca^{2+}]_i$ which reached 2.2 μM final rise in the absence of medium Ca^{2+}, a value which coincided with that obtained in the presence of medium Ca^{2+}. These results indicate that gastrin induces intracellular Ca^{2+} release from the store(s). Therefore, the experiments mentioned below were performed under the conditions of a Ca^{2+}-free medium. The incorporated Ca^{2+}-chelating agent, BAPTA acetoxy methyl ester (50 μM), which enters the cytosol [17], strongly inhibited the gastrin-induced increase in diS-C_3-(5) fluorescence. BAPTA entering the cytosol did not influence the resting diS-C_3-(5) fluorescence. Therefore, intracellular Ca^{2+} released from the store(s) during gastrin stimulation may regulate membrane depolarization.

As shown in fig.3, changes in membrane potential could be recorded in fura-2-loaded cells since there was no interference between the fluorescence from fura-2 and diS-C_3-(5). The gastrin-induced

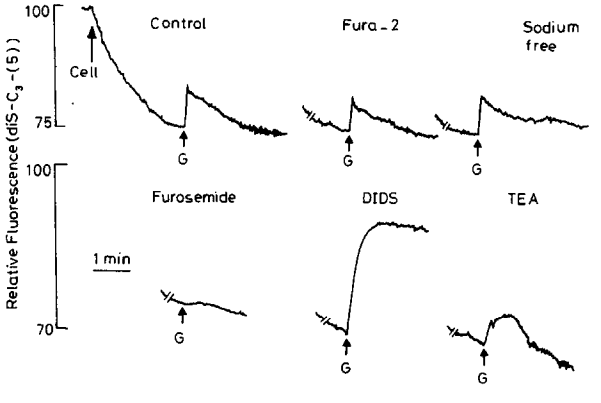

Fig.3. Effects of fura-2, medium Na^+, furosemide (1 mM), DIDS (0.2 mM) and TEA (5 mM) on gastrin-elicited membrane polarization. Pretreatment of cells with the indicated ligands was performed for 3 min at 37°C before gastrin stimulation. Medium Ca^{2+} was zero (0 mM Ca^{2+}, 1 mM EGTA). The trace is representative of at least five similar experiments.

membrane depolarization was strongly inhibited by pretreatment of cells with 1.0 mM furosemide but not with 5.0 mM tetraethylammonium (TEA) and with 2×10^{-4} M 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS). In particular, pretreatment of cells with DIDS caused a rapid but large increase in diS-C_3-(5) fluorescence after gastrin stimulation without inducing the following re-polarization. The omission of medium Na^+ under conditions of equal ionic strength using choline failed to inhibit the gastrin-induced depolarization.

4. DISCUSSION

This study has elucidated the role of Ca^{2+} metabolism during gastrin stimulation under the cascade of steps of the acid secretory process. As shown in previous studies and the present work [1,2], gastrin led to an increase in $[Ca^{2+}]_i$ due to Ca^{2+} released from the store(s), presumably the smooth-surfaced membrane in the apical portion of the gastric parietal cell. Furthermore, gastrin led to membrane depolarization even in the absence of medium Ca^{2+} as measured with diS-C_3-(5). The results suggest that there is a close relationship between intracellular Ca^{2+} metabolism and membrane depolarization during gastrin stimulation. The incorporated Ca^{2+} chelator BAPTA inhibited gastrin-induced membrane depolarization in the absence of medium Ca^{2+}. This in turn suggests that the Ca^{2+} released from store(s) regulates membrane depolarization. BAPTA also inhibited the increase in the fura-2 signal caused by gastrin (not shown). The evidence that Ca^{2+} regulates membrane depolarization was further supported by the fact that in the presence of medium Ca^{2+}, the Ca^{2+} ionophore ionomycin caused a large increase in membrane depolarization without promoting the subsequent re-polarization since no Ca^{2+} gradient was formed [2]. It seems unlikely that the gastrin-elicited depolarization is due to Na^+ entry into the cell from the extracellular space, since the omission of medium Na^+ by substitution with choline had no influence. In contrast, the reduction or omission of medium Na^+ enhanced the gastrin-induced increase in $[Ca^{2+}]_i$, possibly owing to the cessation of Ca^{2+} efflux across the plasma membrane by Na^+-Ca^{2+} antiport [2]. On the other hand, in the

apical surface of the parietal cell a Cl^- conductance is present in the stimulated vesicles but not in the resting state which is distinct from a K^+ conductance [7,8] and could be selectively blocked by furosemide which is regarded as an anion carrier blocker in other systems [18]. K^+ and Cl^- pathways are separable and the Cl^- component in the stimulated state is more severe [7,8]. K^+ diffusion potentials are also present in both resting and stimulated vesicles. The gastrin-induced depolarization was clearly sensitive to furosemide but not TEA which is regarded as a K^+ channel blocker [19], thus suggesting that the Ca^{2+} released from the apical store(s) during gastrin stimulation regulates Cl^- efflux across the luminal surface, possibly via (a) Cl^- channel(s), which occur(s) separately from K^+ efflux, leading to membrane depolarization. The anion exchanger, i.e. Cl^--HCO_3^- exchanger, of the parietal cell is inhibited by DIDS [20]. A secondary or indirect effect of stimulation seems to be increased Cl^- uptake across the basolateral membrane via Cl^--HCO_3^- exchange [7]. If DIDS acts inhibitorily upon a Cl^--HCO_3^- exchanger, the failure of Cl^- replenishment could occur resulting in a significant loss of cellular Cl^- by the gastrin-induced Cl^- efflux across the luminal surface and in enhancement of gastrin-elicited membrane depolarization.

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