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Gastrin induces intracellular Ca^{2+} release and acid secretion regulation by the microtubular-microfilamentous system

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In the absence of extracellular Ca^{2+} gastrin induced accumulation of amino [^{14}C]pyrine as an index of acid secretion and evoked a cytosolic Ca^{2+} rise (measured by quin2/AM), suggesting that an intracellular Ca^{2+} release after the activation of the gastrinergic pathway may trigger acid secretion. In isolated parietal cells pretreatment with colchicine or cytochalasin B abolished the amino [^{14}C]pyrine accumulation and the rise in cytosolic Ca^{2+} concentration evoked by gastrin. The results suggest that the microtubular-microfilamentous system regulates gastrin-induced intracellular Ca^{2+} release and acid secretion.

In the process of acid secretion from parietal cells, cyclic AMP synthesis is a necessary intermediate event in the histaminergic pathway [1]. In contrast, cholinergic stimulation requires the presence of extracellular Ca^{2+} and causes Ca^{2+} influx from the extracellular space [2–4]. The second messenger of the gastrinergic pathway, however, is as yet unknown, although the possibility has been documented that gastrin may induce Ca^{2+} release from intracellular stores [3,5,6]. On the other hand, it has been demonstrated that the microtubular-microfilamentous system participates in the process of acid secretion since colchicine, vincristine and cytochalasin B could abolish the acid secretion [7,8]. The function of the microtubular-microfilamentous system including tubulovesicular elements and secretory canaliculi may be regulated by intracellular Ca^{2+} [9].Therefore, in this study the possible interactions between the intracellular Ca^{2+} release and the function of the microtubular-microfilamentous system in the parietal cell during the stimulation of gastrin have been examined.

Dispersed mucosal cells from guinea pig gastric mucosa (Hartley, male, 350 g weighing) were prepared by the digestion by collagenase (0.01%) and dispase (0.08%) in tissue culture medium (RPMI 1640) containing 10% fetal calf serum and by brief mechanical disruption in EDTA (0.5 mM) in Ca^{2+}-Mg^{2+} free Krebs-Ringer bicarbonate buffer as previously described [5,10]. Then dispersed mucosal cells containing parietal, chief, mucous neck, somatostatin-producing and other endocrine cells were loaded into the Beckman elutriation system [10]. A parietal cell enriched fraction was obtained with a flow rate of 48 ml/min and at centrifugal speed of 1450 rpm. Parietal cells were identified by their large size (diameter > 18 um) and their eosinophilic cytoplasm [11]. The abundance of parietal cells was 85%. Cell viability of parietal cells was determined by exclusion of 0.4% Trypan blue. After the experiments (described below) almost 80% of the cells were viable.

The index of acid secretion was monitored by the method of amino [^{14}C]pyrine accumulation

Abbreviations: EDTA, ethylenediamine tetraacetic acid; Quin2/AM, 2-[2-amino-5-methylphenoxy]methyl-6-methoxy-8-aminoquinoline-*N*,*N*,*N'*,*N'*-tetraacetic acid tetraacetoxymethyl ester.

TABLE I

THE EFFECTS OF CYTOCHALASIN B AND COLCHICINE ON GASTRIN-INDUCED AMINO[^{14}C]PYRINE ACCUMULATION IN THE ABSENCE OF EXTRACELLULAR Ca^{2+}

10^6 parietal cells were suspended in Ca^{2+}-Mg^{2+} free Krebs-Ringer bicarbonate buffer with 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes) buffer, 0.2% bovine serum albumin and 0.2% glucose. In all experiments (Table I and Table II), the Ca^{2+}-free medium was prepared by omitting $CaCl_2$ and by adding 1.0 mM EDTA in Krebs-Ringer bicarbonate buffer. Mg^{2+} in addition to Ca^{2+} was omitted from the medium. 1 ml of cell suspension was preincubated with or without indicated agents (10 μg/ml cytochalasin B (CB), 10 μg/ml colchicine (COL)) for 20 min at 37°C in an atmosphere of 95% O_2/5% CO_2 (pH 7.4). Then further incubation was done with or without of 10^{-7} M human gastrin-17 (HG-17) plus 0.1 μCi amino[^{14}C]pyrine for 15 min at 37°C. At the end of the incubation period, the cell suspension was placed directly on a Millipore filter (RAWP, 1.2 μm) under mild suction and immediately washed four times with 3 ml of ice-cold stop solution which was basically the same as incubation buffer. The filter pads were digested by 500 μl NCS and 200 μl distilled water for 12 h. After adding 8 ml of Aquasol samples were counted in a Packard liquid scintillation spectrometer. The amino[^{14}C]pyrine trapped into the cells (10^6 parietal cells) was calculated as the percentage of total amino[^{14}C]pyrine present in the cells plus the medium (percentage of total). 100% (control) corresponds to the percentage of total in non-stimulated cells. Each value represents mean ± S.E. from three to eight separate experiments. Significant difference from human gastrin-17 alone (*$P < 0.05$) by unpaired *t*-test calculated from percentage of total.

Agents added	Amino[^{14}C]pyrine accumulation	
	percentage of total	percentage of control
Control	1.35 ± 0.33 *	100
HG-17	2.45 ± 0.29	181.9
HG-17 + CB	1.43 ± 0.08 *	106.3
HG-17 + COL	1.60 ± 0.10 *	119.2

[12] (see Table I), and the measurement of cytosolic Ca^{2+} was determined by the method of quin2/AM loading [13] (see Table II).

Table I shows the amino [^{14}C]pyrine accumulation during 15 min under stimulation by 10^{-7} M gastrin in the absence of extracellular Ca^{2+}. Gastrin led to an accumulation of amino [^{14}C]pyrine exceeding that in non-stimulated cells by a factor of 1.8. The amount of accumulated amino[^{14}C]pyrine due to gastrin addition was almost equal when it was measured in the presence of extracellular Ca^{2+}

TABLE II

THE EFFECTS OF CYTOCHALASIN B AND COLCHICINE ON GASTRIN-INDUCED CYTOSOLIC Ca^{2+} RISE IN THE ABSENCE OF EXTRACELLULAR Ca^{2+}

The cytosolic free Ca^{2+} concentration was measured by the method previously described [13]. Quin2/AM (100 μM) was added to cell suspension ($5 \cdot 10^6$ cells/ml) in medium-199 containing 20 mM Hepes and 0.2% bovine serum albumin and incubated for 20 min, the subsequently diluted 5-fold with the same medium in order to lower the ester concentration. Further incubation was done for 40 min at 37°C in an atmosphere of 95% O_2/5% CO_2 (pH 7.4). After loading of quin2/AM, rinsed cell suspension (10^6 cells) was resuspended in 1 ml of Ca^{2+}-Mg^{2+} free Krebs-Ringer bicarbonate buffer containing 10 mM Hepes, 0.2% bovine serum albumin and 0.2% glucose. Cell suspension was preincubated with or without indicated agents (10 μg/ml cytochalasin B (CB), 10 μg/ml colchicine (COL)) for 20 min at 37°C in an atmosphere of 95% O_2/5% CO_2. Then further incubation (15 min, 37°C) was done with or without of 10^{-7} M human gastrin-17 (HG-17) in a Ca^{2+}-free high K^+ medium (Ca^{2+}; 0 mM, K^+; 108 mM, Na^+; 40.6 mM in Krebs-Ringer bicarbonate buffer). For measurement of fluorescence, 1 ml of cell suspension was centrifuged for a few second at 10000 rpm and the cells were resuspended in 2 ml of Krebs-Ringer bicarbonate buffer and transferred to the cuvette. Quin2/AM fluorescence was recorded with Hitachi 650-10 LC fluorescence spectrometer. The excitation and emission wavelengths were 339 and 492 nm with 4 and 10 nm bandwidths. Fluorescence readings are presented in arbitrary units. Cytosolic Ca^{2+} concentration was calculated using the following formula [13], $[Ca^{2+}]_i = 115 \text{ nM } (F - F_{min})/(F_{max} - F)$. Cells were lysed with sonication and fluorescence at high Ca^{2+} (1.3 mM Ca^{2+}, F_{max}) and low Ca^{2+} (2 mM EGTA, F_{min}) was determined. F is the relative fluorescence measurement of the sample. 100% (control) corresponds to the fluorescence in non-stimulated cells. Quin2/AM was stocked 10 mM in dimethyl sulfoxide to a final concentration of 100 μM. Each value represents mean ± S.E. from three to nine separate experiments. Significant difference from human gastrin-17 alone (*$P < 0.05$) by unpaired *t*-test calculated from arbitrary unit.

Agents added	Cytosolic Ca^{2+} level	
	$[Ca^{2+}]_i$ (nM)	percentage of control
Control	172.5 ± 4.7 *	100
HG-17	254.1 ± 6.9	147.3
HG-17 + CB	145.4 ± 5.8 *	84.3
HG-17 + COL	178.2 ± 18.4 *	103.3

(percentage of total: Ca^{2+}-free, 2.45 ± 0.29 ($n = 6$); 1.3 mM Ca^{2+}, 2.09 ± 0.14 ($n = 6$)), indicating that gastrin-induced acid secretion is independent of the presence of extracellular Ca^{2+}. Amino-[^{14}C]pyrine accumulation in non-stimulated cells

was almost equal in the presence or absence of extracellular Ca^{2+}, too. In the absence of extracellular Ca^{2+}, parietal cells pretreated with 10 μg/ml cytochalasin B or 10 μg/ml colchicine failed to show the effect of gastrin-induced amino[^{14}C]-pyrine accumulation. Yet the rise in cytosolic Ca^{2+} concentration by gastrin (10^{-7} M) addition was found in a Ca^{2+}-free medium (from 172.5 ± 4.7 nM ($n = 9$) to 254.1 ± 6.9 nM ($n = 6$)) and coincided in time with the gastrin-induced amino[^{14}C]-pyrine accumulation (Table II). In the presence of extracellular Ca^{2+}, gastrin could evoke the rise in cytosolic Ca^{2+} concentration (percentage compared to non-stimulated cells, 216.0 ± 3.0 ($n = 5$)) only when extracellular Na^+ was replaced by 40.6 mM choline$^+$ (Ca^{2+}; 2.6 mM, K^+; 108 mM, Na^+; 0 mM). The cytosolic Ca^{2+} rise by gastrin in a Ca^{2+}-free medium was almost 40% that occurring in the presence of extracellular Ca^{2+}. Arbitrary units of fluoresence by quin2/AM in non-stimulated cells were almost equal in the presence or absence of extracellular Ca^{2+}. The partial increase in cytosolic Ca^{2+} elicited by gastrin in a Ca^{2+}-free medium suggests that gastrin acts on the release of Ca^{2+} from an intracellular organelle. In contrast, the rise in cytosolic Ca^{2+} concentration by cholinergic stimulation disappeared in the absence of extracellular Ca^{2+}, indicating that the action of cholinergic stimulation is mainly due to influx of Ca^{2+} from the extracellular space [4–6]. Cytochalasin B and colchicine could also eliminate the cytosolic Ca^{2+} rise due to gastrin, suggesting that the intracellular Ca^{2+} release by gastrin might be regulated by the microtubular-microfilamentous system (Table II).

In the previous study [5] it has been demonstrated that the ionophore A23187 mimicked the gastrin-evoked acid secretion in showing a biphasic pattern. The two phases differed in their requirement for extracellular Ca^{2+}, that is, the first phase was independent of extracellular Ca^{2+}, whereas the second one was not, indicating that the initial step of acid secretion evoked by ionophore A23187 may be caused by Ca^{2+} release from an intracellular store. This was further substantiated by the fact that the ionophore A23187 could increase the cytosolic Ca^{2+} concentration in a Ca^{2+}-free medium. In this phase colchicine and cytochalasin B caused an inhibition of ionophore A23187-induced cytosolic Ca^{2+} rise. The requirement for acid secretion in vitro and in vivo can be differentiated into one for extracellular and one for intracellular Ca^{2+} [14,15]. Cytochalasin B did not affect the second phase of acid secretion which required extracellular Ca^{2+} [7]. This makes it is unlikely that the observed effects is due to deprivation of glucose [16] or an alteration of a membrane receptor [17]. In conclusion gastrin induces intracellular Ca^{2+} release at the initial stage of acid secretion, and this Ca^{2+} mobilization is regulated by the microtubular-microfilamentous system.

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