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A novel effect of rebamipide: generation of $[Ca^{2+}]_i$ oscillations through activation of CCK_1 receptors in rat pancreatic acinar cells

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Abstract

The protective effect of 2-(4-chlorobenzoylamino)-3-[2(1*H*)-quinolinon-4-yl]-propionic acid (rebamipide) on gastric mucosa is well established. Here we demonstrate that rebamipide acts on pancreatic acinar cells to generate oscillations of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) through the activation of cholecystokinin subtype 1 (CCK_1) receptors. At concentrations higher than 5 μM, rebamipide induced $[Ca^{2+}]_i$ oscillations in individual fura-2-loaded pancreatic acinar cells. The frequency of oscillations increased with increasing concentrations of rebamipide, while the latency between stimulation of cells and initiation of $[Ca^{2+}]_i$ oscillations decreased with increasing concentration. The $[Ca^{2+}]_i$ oscillations evoked by rebamipide were inhibited by the CCK_1 receptor antagonist L-364,718 but not by atropine or the CCK_2 receptor antagonist L-365,260 indicating that rebamipide is a nonpeptide CCK_1 receptor agonist.

Keywords: Rebamipide; CCK_1 receptor; $[Ca^{2+}]_i$ oscillation; Pancreatic acinar cell

1. Introduction

2-(4-Chlorobenzoylamino)-3-[2(1*H*)-quinolinon-4-yl]-propionic acid (rebamipide) is an anti-ulcer agent, which has been approved in some Asian countries, for therapeutic use in patients. The protective effect of rebamipide on gastric mucosa was first reported by Ishiyama et al. (1985), and has subsequently been confirmed by a number of investigators (Ogino et al., 1992; Yoshikawa et al., 1993; Suzuki et al., 1994; Kim and Hong, 1995, 1997). Rebamipide appears to exert its cytoprotective effect by increasing prostaglandin generation in gastric mucosa and by scavenging reactive oxygen species and inhibiting their production (Yamasaki et al., 1987; Ogino et al., 1992; Iinuma et al., 1998). In addition to this protective effect on gastric mucosa, rebamipide also ameliorates hepatic dysfunction induced by ischemia/reperfusion and attenuates diabetic nephropathy (Lee and Kim, 1995; Ha et al., 1997).

We are currently studying the role of oxidative stress in the pathogenesis of acute pancreatitis and the protective effect of rebamipide against this disease. During the course of this study, we unexpectedly observed that rebamipide evokes oscillations of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in rat pancreatic acinar cells. Since these oscillations were inhibited by L-364,718, but not by atropine or L-365,260, activation of cholecystokinin subtype 1 (CCK_1) receptors is thought to be responsible for the rebamipide-evoked $[Ca^{2+}]_i$ oscillations.

2. Materials and methods

Male Sprague–Dawley rats (150–250 g) were killed by decapitation and the pancreata were immediately removed and trimmed of fat on ice. The acini were prepared using 50 U/ml collagenase by a slight modification of the methods described previously (Matozaki et al., 1990; Toescu et al., 1993). The isolated pancreatic acinar cells were suspended in a HEPES-buffered physiological solu-

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tion containing (in mM): NaCl 104, KCl 4.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 0.5, HEPES–Na 25, HEPES free acid 15, D-glucose 15, Minimal Eagle's Medium (MEM) amino acids (×1), 1% bovine serum albumin, soybean trypsin inhibitor (4 mg/ml) and L-glutamine 2 (adjusted to pH 7.4, gassed with 100% O_2).

Cells were loaded with fura-2 by incubation with 2 μM acetoxymethyl ester of fura-2 (fura-2/AM) in HEPES buffered solution equilibrated with 100% O_2 for 40 min at room temperature. They were washed twice and resuspended in a HCO_3^--buffered solution containing (in mM): NaCl 110, KCl 4.5, NaH_2PO_4 1.0, $MgSO_4$ 1.0, $CaCl_2$ 1.5, $NaHCO_3$ 25, HEPES–Na 5, HEPES free acid 5, D-glucose 10 and equilibrated with 95% O_2, 5% CO_2 to give a pH of 7.4. The cells were allowed to attach to a coverslip which formed the base of a cell chamber mounted on the stage of inverted microscope and were superfused with the HCO_3^--buffered solution at a flow rate of 2 ml/min. The buffer was continuously gassed with 5% CO_2 in O_2 during experiments and a water-jacketed perfusion line maintained the temperature of the perfusate at 37°C between the pump and the cell chamber. $[Ca^{2+}]_i$ was measured by spectrofluorometry (Photon Technology International, Brunswick, NJ) with excitation at 340 and 380 nm and emission measured at 510 nm. The values of $[Ca^{2+}]_i$ were calculated from the ratio of fluorescence intensities ($F_{340/380}$) according to Grynkiewicz et al. (1985).

Collagenase (type IV), soybean trypsin inhibitor, HEPES, bovine serum albumin, MEM amino acids, glutamine and atropine were purchased from Sigma (St. Louis, MO). Fura-2/AM was obtained from Molecular Probes (Eugene, OR). L-364,718 and L-365,260 were provided from ML Laboratories (Liverpool, UK). Rebamipide was supplied from Korea Otsuka Pharmaceutical (Seoul, Korea) and was directly dissolved in HCO_3^--buffered solution just before each experiment was performed. Rebamipide did not dissolve completely at concentrations higher than 1 mM and, thus, 500 μM was the maximum concentration used in this study.

3. Results

Rebamipide (5–500 μM) evoked $[Ca^{2+}]_i$ oscillations in isolated rat pancreatic acinar cells. The nature of these oscillations was similar to those induced by physiological concentrations of CCK (5–20 pM), namely repetitive discrete $[Ca^{2+}]_i$ transients arising from a constant basal level (Fig. 1). The frequency of oscillations was dependent on the concentration of rebamipide: 0.28 ± 0.06/min at 5 μM ($n = 3$), 0.67 ± 0.09/min at 50 μM ($n = 7$) and 1.47 ± 0.20/min at 500 μM ($n = 6$). The time lag between stimulation of cells and initiation of Ca^{2+} response decreased with increasing rebamipide concentration, being 10.5 ± 0.1 min at 5 μM, 4.4 ± 0.5 at 50 μM and 1.4 ± 0.4 min at 500 μM. The amplitude of spikes was not significantly affected by rebamipide concentration between 5 and 500 μM.

Since $[Ca^{2+}]_i$ oscillations are known to be generated by the activation of both CCK_1 receptors and muscarinic

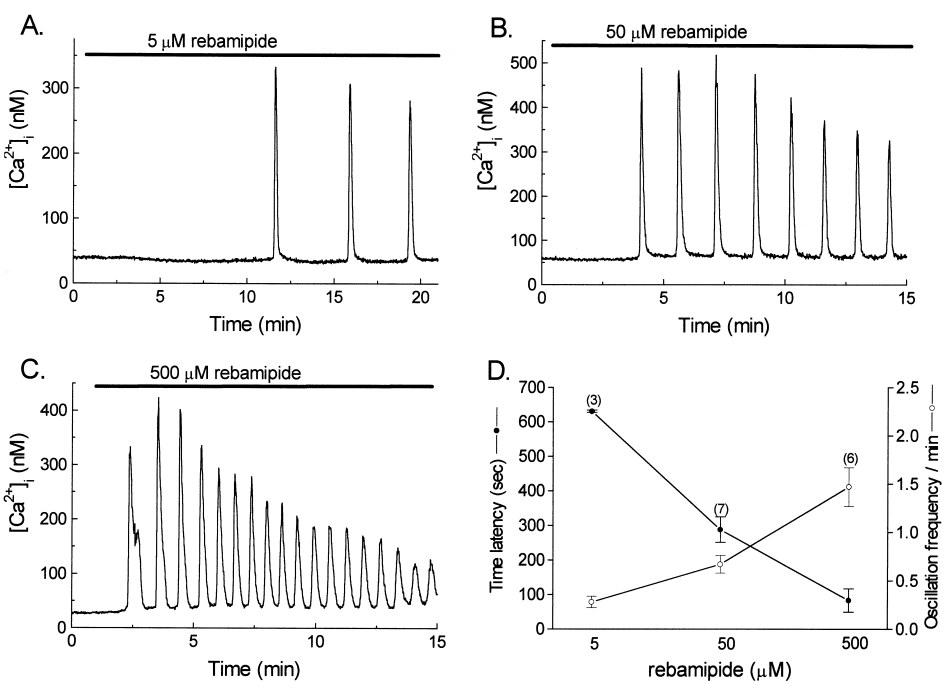

Fig. 1. Effect of various concentrations of rebamipide on changes in $[Ca^{2+}]_i$ in rat pancreatic single acinar cells. (A, B and C) Each panel is representative of three to seven separate experiments. (D) Frequency of $[Ca^{2+}]_i$ oscillations evoked by rebamipide and latency between stimulation of cells and initiation of response. Data are means ± S.E.M. Note the different scales.

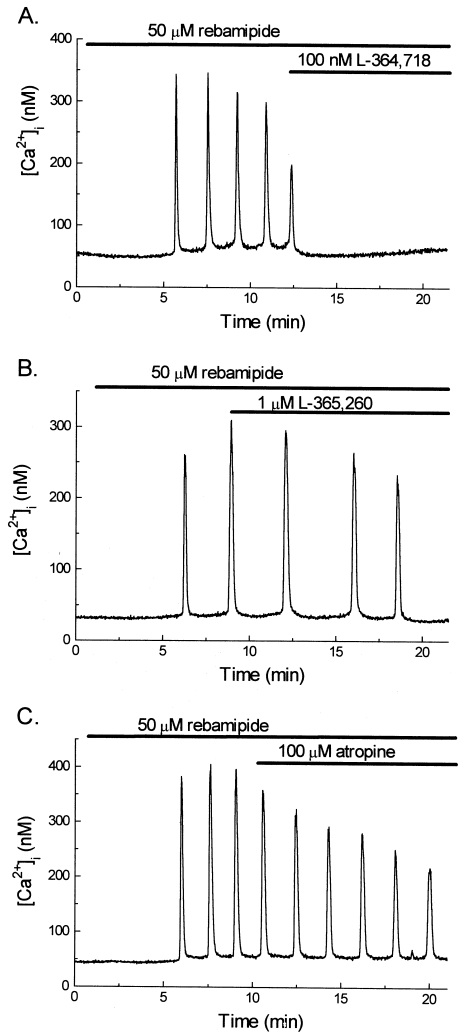

Fig. 2. Effect of L-364,718 (A), L-365,260 (B) and atropine (C) on rebamipide-induced $[Ca^{2+}]_i$ oscillations in rat pancreatic single acinar cells. Data are representatives of three to seven separate experiments.

receptors in pancreatic acinar cells (Osipchuk et al., 1990; Tsunoda et al., 1990), specific antagonists were used to investigate which receptor was responsible for the generation of rebamipide-evoked $[Ca^{2+}]_i$ oscillations. The $[Ca^{2+}]_i$ oscillations were completely and irreversibly inhibited by the CCK_1 receptor antagonist L-364,718 ($n = 7$; Fig. 2A) but not by L-365,260 (a CCK_2 receptor specific antagonist) ($n = 6$; Fig. 2B). The muscarinic receptor antagonist atropine also failed to inhibit the rebamipide-evoked $[Ca^{2+}]_i$ oscillations ($n = 3$; Fig. 2C).

4. Discussion

In pancreatic acinar cells, physiological concentrations of CCK (5–20 pM) and acetylcholine (50–100 nM) evoke $[Ca^{2+}]_i$ oscillations which are believed to be the main intracellular signals for enzyme and fluid secretion (Kanno, 1998). These two agonists evoke different types of $[Ca^{2+}]_i$ oscillations (Berridge, 1990; Yule et al, 1991). Acetylcholine usually induces sinusoidal oscillations which are regular fluctuations of $[Ca^{2+}]_i$ with high frequency from a mean elevated level. By contrast, CCK evokes discrete transient elevations in $[Ca^{2+}]_i$ arising from a constant resting level.

In our experiments, as shown in Fig. 1, rebamipide evoked transient $[Ca^{2+}]_i$ oscillations at each of the three concentrations we used; sinusoidal oscillations were never observed. Therefore, the oscillatory pattern observed during rebamipide stimulation displayed characteristics typical of CCK-evoked $[Ca^{2+}]_i$ oscillations. This suggested that rebamipide might exert its effect by binding to the CCK receptors. But there are alternative explanations. First, since rebamipide penetrates the cell membrane, some down-stream elements in the G-protein-coupled signal transduction pathway, rather than receptor itself, might be the primary target of rebamipide action. Second, although the CCK receptor is best known initiator of this type of transient $[Ca^{2+}]_i$ oscillations in pancreatic acinar cells, the involvement of other receptors could not be ruled out. For example, acetylcholine has also been reported to evoke transient $[Ca^{2+}]_i$ oscillations in certain conditions such as high resting $[Ca^{2+}]_i$ (Toescu et al., 1993). However the effects of receptor-specific antagonists clearly demonstrate that activation of CCK_1 receptor is the principal mechanism for the generation of $[Ca^{2+}]_i$ oscillations.

In conclusion, rebamipide induces $[Ca^{2+}]_i$ oscillations through activation of CCK_1 receptors in rat pancreatic acinar cells. The pharmacological properties of rebamipide as a CCK_1 receptor agonist are currently under investigation.

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