Vol. 137, No. 1, 1986 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 29, 1986 Pages 281-285

INCREASES IN CELLULAR CALCIUM CONCENTRATION STIMULATE PEPSINOGEN SECRETION FROM DISPERSED CHIEF CELLS

J-P. Raufman, S. Berger, L. Cosowsky, and E. Straus

Division of Digestive Diseases, Department of Medicine, State University of New York Health Sciences Center at Brooklyn, Brooklyn, NY 11203

Received April 11, 1986

SUMMARY Intracellular calcium concentration ([Ca]i) and pepsinogen secretion from dispersed chief cells from guinea pig stomach were determined before and after stimulation with calcium ionophores. [Ca]i was measured using the fluorescent probe quin2. Basal [Ca]i was 105±4 nM. Pepsinogen secretion was measured with a new assay using ^{125}I-albumin substrate. This assay is 1000-fold more sensitive than the widely-used spectrophotometric assay, technically easy to perform, rapid, and relatively inexpensive. The kinetics and stoichiometry of ionophore-induced changes in [Ca]i and pepsinogen secretion were similar. These data support a role for calcium as a cellular mediator of pepsinogen secretion.

INTRODUCTION Several investigators have used indirect evidence to propose that a change in [Ca]i is one mechanism that mediates secretagogue-induced pepsinogen secretion (1-3). Recently, an isolated chief cell preparation (4) and fluorescent calcium probes such as quin2 have become available (5-6), thereby permitting direct measurement of changes in [Ca]i.

In the present study, we related changes in [Ca]i, measured with quin2, to changes in pepsinogen secretion from dispersed chief cells. Because the commonly-used spectrophotometric assay for pepsin (7-8) is not sensitive enough to determine pepsinogen secretion from dispersed chief cells and the more sensitive [^{14}C]-methemoglobin assay (1,4,9) is costly (>$1/assay plus costs of scintillation fluid and radioactive waste disposal) and difficult to prepare, we developed a pepsin assay using ^{125}I-albumin substrate. This assay is 1000-fold more sensitive than the spectrophotometric assay and can be used to measure pepsinogen secretion from dispersed chief cells.

MATERIALS AND METHODS Male Hartley guinea pigs (150 g) were obtained from Marland Farms, Hewitt, NJ; bovine albumin (fraction V), porcine pepsinogen (grade 1, lot 98C-0450, 2980 peptic units/mg) from Sigma Chemical, St. Louis, MO; Sephadex G-50 from Pharmacia, Piscataway, NJ; A23187, ionomycin, quin2 and quin2/AM from Calbiochem, LaJolla, CA; and Na ^{125}I (100 mCi/ml) from Amersham Corp., Arlington Hts., IL. All other chemicals used were of the highest purity commercially available.

Abbreviations: [Ca]i, intracellular free calcium concentration; quin2/AM, the acetoxymethyl derivative of quin2.

0006-291X/86 $1.50

Dispersed chief cells from guinea pig stomach were prepared and pepsinogen secretion was determined as described previously (4). ^{125}I-albumin (specific activity, 124 uCi/ug) was prepared according to the method of Hunter and Greenwood (10). A single preparation of ^{125}I-albumin was adequate for the performace of approximately 1,500 assays and was stable for at least 4 months. Pepsin stock solutions, prepared by dissolving pepsinogen in 0.005M acetate buffer (pH 4.0), were stored at 4°C. Pepsin standards were prepared daily from serial dilutions of the stock solution in water. Samples (100 ul) were added to 400 ul 50 mM glycine buffer (pH 3.0) in 1.8 ml Eppendorf tubes at 4° C. Immediately after adding 100 ul ^{125}I-albumin (25,000 cpm/100 ul), the tubes were incubated and enzymatic activity was terminated by adding 200 ul 1% (wt/vol) bovine albumin and 400 ul 4.5% (vol/vol) tricholoroacetic acid. After 10 min at 4°C, the tubes were centrifuges in a Beckman Microfuge B at 10,000 g for 2 min. Radioactivity was determined in the supernatant (500 ul).

For determination of [Ca]i, chief cells (10^7 cells/ml) were suspended in standard incubation solution (4) containing 1.0 mM $MgCl_2$, 1.5 mM $CaCl_2$, and 20 uM quin2/AM and incubated for 20 min in a shaking metabolic incubator (60 cycles /min) at 37 °C with 100% oxygen. Cells were then centrifuged (10,000 g x 15 sec) and resuspended in incubation solution containing 1.5 mM $CaCl_2$ (2 x 10^6 cells/ml) at 37°C in a Perkin-Elmer spectrofluorimeter. Excitation and emission wavelengths were 339 nm with 4 nm slits and 492 nm with 10 nm slits, respectively. [Ca]i was calculated as [Ca]i=Kd(F-Fmin)/(Fmax-F), where Kd (115) is the apparent dissociation constant quin2 for calcium; F is the fluorescence of intact quin-2 loaded cells; Fmax is the fluorescence of quin2 in the presence of saturating calcium concentrations determined after cell solubilization with 0.02% Triton X-100; and Fmin is the fluorescence of quin2 when it is not bound to calcium (5).

RESULTS To determine the proper conditions for the ^{125}I-albumin pepsin assay, we examined the effects of changes in time, pH, and trichloroacetic acid concentration on the radioactivity released to the supernatant with a fixed concentration of pepsin. (Fig. 1) The rate of hydrolysis as

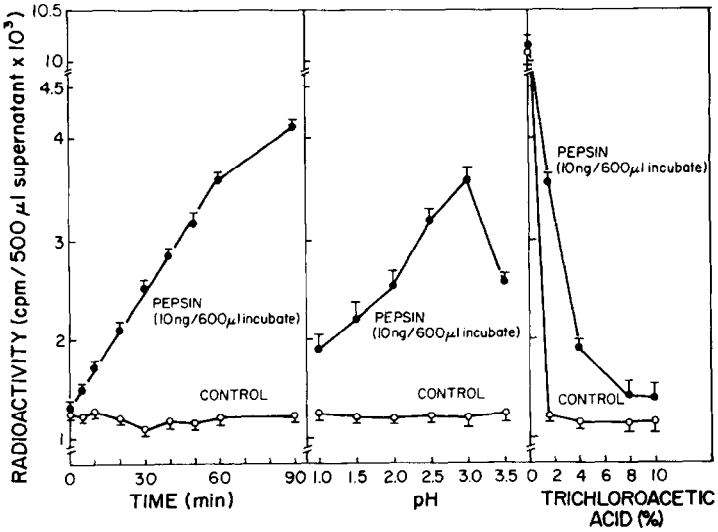
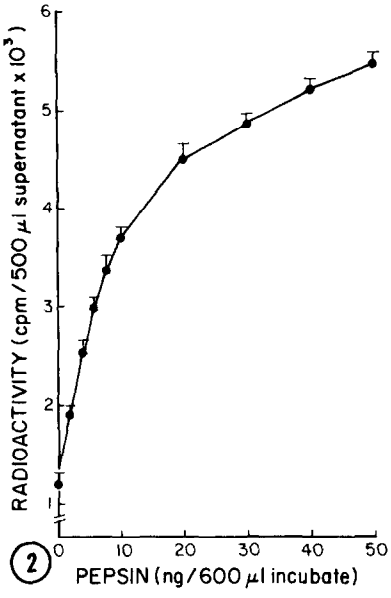


Figure 1. Effect of incubation time (left), pH (middle), and tricholoracetic acid concentration (right) on ^{125}I-albumin pepsin assay. In each experiment each value was determined in duplicate and results given are means ± SE from 3 experiments.

determined by release of radioactivity to the supernatant was constant for the first 60 min of incubation with 10 ng pepsin and decreased over the susbsequent 30 min of incubation. (Fig. 1, left) Radioactivity released to the supernatant in the presence of 10 ng pepsin/600 ul incubate increased as the pH was increased from 1 to 3 and decreased at higher pH. (Fig. 1, middle) Radioactivity released to the supernatant with and without added pepsin decreased as the final concentration of trichloroacetic acid was increased from 0 to 10%. (Fig. 1, right) Subsequent pepsin assays were for 60 min at pH 3 with a final trichloroacetic acid concentration of 1.5%. Radioactivity released to the supernatant was linear as the pepsin concentration was increased from 1 to 8 ng/600 ul incubate. (Fig 2) In subsequent experiments appropriate dilutions were made to maintain values on the linear portion of the curve.

Basal [Ca]i was 105 ± 4 nM (mean ± SE of 21 separate observations). Both calcium ionophores tested caused a >10 - fold increase in [Ca]i and pepsinogen secretion. (Fig. 3) The kinetics of A23187- and ionomycin-induced changes in [Ca]i and pepsinogen secretion were similar. That is, there was a rapid increase in [Ca]i and pepsinogen secretion within



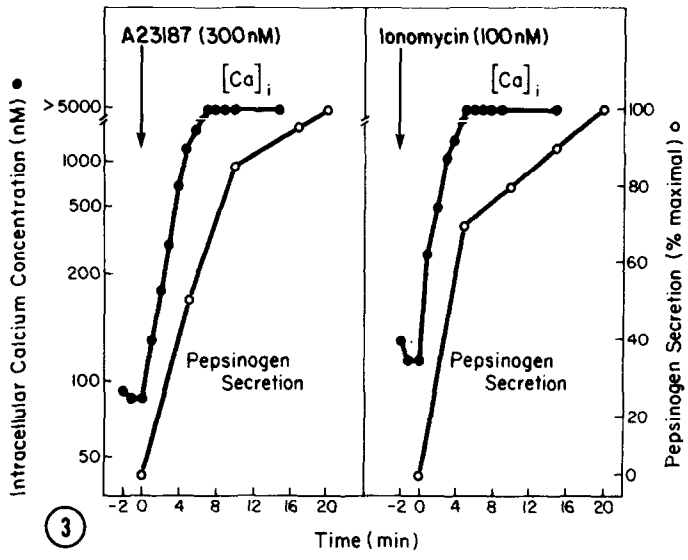


Figure 2. Effect of increasing concentration of pepsin on hydrolysis of ^{125}I-albumin. In each experiment each value was determined in duplicate, and results given are means ± SE from 3 experiments.

Figure 3. Effects of A23187 and ionomycin on [Ca]i and pepsinogen secretion from dispersed chief cells. Ionophores were added at the times indicated by arrows. [Ca]i and pepsinogen secretion were determined at the times indicated. Pepsinogen secretion at a given time is expressed as the percentage of the response at 20 min. In each experiment each value was determined in singlicate for [Ca]i and duplicate for pepsinogen secretion, and results given are means from at least 3 separate experiments.

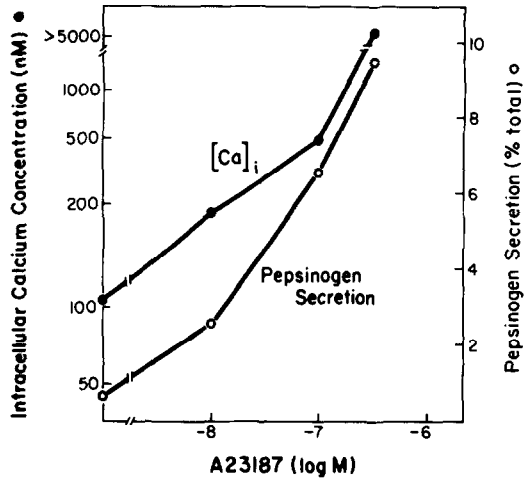


Figure 4. Dose-response curves for effects of A23187 on [Ca]i and pepsinogen secretion from dispersed chief cells. [Ca]i and pepsinogen secretion with the indicated concentration of A23187 were determined after 20 min incubation. Pepsinogen secretion was calculated as a percentage of pepsinogen in cells at beginning of incubation that was released in extracellular medium during incubation. In each experiment each value was determined in singlicate for [Ca]i and duplicate for pepsinogen secretion, and results given are means from at least 3 separate experiments.

the first minutes of stimulation with a subsequent decrease in the rate of pepsinogen secretion after 10 min incubation. With both ionophores, quin2 was saturated with calcium after a 5-min incubation. (Fig. 3) Effective concentrations for A23187-induced changes in pepsinogen secretion and [Ca]i were similar. (Fig. 4) This similarity of dose-response curves was also observed with ionomycin (data not shown). Concentrations of ionophore greater than 300 nM were not tested because of possible cell damage at higher concentrations (4,11).

DISCUSSION The pepsin assay described in this paper has several advantages over previously described methods when used to determine pepsinogen secretion from *in vitro* tissue preparations. Most important is its greater sensitivity. Whereas spectrophotometric assays using unlabeled hemoglobin or albumin substrate are sensitive to micrograms of pepsin (7,8), the ^{125}I-albumin radioassay is sensitive to nanograms of pepsin. Although use of [^{14}C]-methemoglobin substrate increases the sensitivity of the pepsin assay (1,4,9), this assay has not been characterized as well and is more expensive. A less sensitive fluorometric assay (12) is more difficult to perform and requires a 16-hour incubation. Radioimmunoassay for pepsinogen requires 4 days incubation with two antisera making this method impractical for studying pepsinogen secretion (13).

The present data indicate that fluorescent calcium probes can be used to measure changes in [Ca]i in dispersed chief cells from guinea pig stomach. The basal [Ca]i determined for chief cells is similar to that for parietal cells (14) and pancreatic acinar cells (11). Moreover, increases in [Ca]i are associated with increases in pepsinogen secretion from dispersed chief cells. The kinetics and stoichiometry of ionophore-induced changes in [Ca]i and pepsinogen secretion are similar. These findings support earlier indirect observations suggesting a potential role for cellular calcium as a mediator of secretagogue-induced pepsinogen secretion (1-3).

ACKNOWLEDGEMENTS This research was supported by NIH Grant AM-34189. Dr. Raufman is a recipient of an AGA/Merrell-Dow Research Scholar Award.

REFERENCES

1. Raufman, J-P., Kasbekar, D.K., Jensen, R.T., and Gardner, J.D. (1983) Am. J. Physiol. 245, G525-G530.
2. Koelz, H.R., Hersey, S.J., Sachs, G., and Chew, C.S. (1982) Am. J. Physiol. 243, G218-G225.
3. Hersey, S.J., Norris, S.H. and Gibert, A.J. (1984) Ann. Rev. Physiol. 46, 393-402.
4. Raufman, J-P., Sutliff, V.E., Kasbekar, D.K., Jensen, R.T. and Gardner, J.D. (1984) Am. J. Physiol. 247, G95-G104.
5. Tsien, R.Y., Pozzan, T. and Rink, T.J. (1982) J. Cell. Biol. 94, 325-334.
6. Tsien, R.Y., Pozzan, T. and Rink, T.J. (1982) Nature 295, 68-71.
7. Anson, M.L., and Mirsky, A.E. (1932) J. Gen. Physiol. 16, 59-63.
8. Schlamowitz, M. and Peterson, L.U. (1959) J. Biol. Chem. 234, 3137-3145.
9. Kasbekar, D.K., Jensen, R.T., and Gardner, J.D. (1983) Am. J. Physiol. 244, G392-G396.
10. Hunter, W.M. and Greenwood, F.C. (1962) Nature London. 194, 495-496.
11. Ochs, D.L., Korenbrot, J.I., and Williams, J.A. (1985) Am. J. Physiol. 29, G389-G-398.
12. Ford, T.F., Hermon-Taylor, J., and Grant, D.A.W. (1982) Clin. Chim. Acta. 126, 17-23.
13. Samloff, I.M. (1982) Gastroenterology 82, 26-33.
14. Muallem, S. and Sachs, G. (1985) Am. J. Physiol. 248, G216-G228.