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Influence of Calcium on the Release of Gastrin from Isolated Rodent G Cells (41113)

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Abstract. A fraction of isolated and enriched rodent, gastrin-containing G cells was prepared using a previously described technique (1) and employed to investigate the role of calcium in gastrin release *in vitro*. Incubation of the enriched G cells in medium containing low extracellular calcium resulted in a significant, 20–40% increase in basal gastrin release. It was determined that hormone secretion is enhanced when the cells are incubated under basal conditions in medium containing low levels of calcium (0–0.3 m*M* $CaCl_2$) and inhibited when the extracellular calcium concentration is increased above 2.4 m*M*. Addition of Verapamil, a drug which prevents calcium entry into isolated cells and accelerates calcium efflux, to the medium at a final concentration of 10 μM, induced a significant 2–2.5 fold increase in gastrin release. Peptone-stimulated gastrin release was not influenced by either Verapamil or incubation of the cells in calcium-free medium. The results suggest that inhibition of calcium influx or acceleration of calcium efflux from the G cell may be a critical step in the initiation of gastrin release.

The function of many exocrine and endocrine tissues has been demonstrated to be dependent on the intracellular concentration of ionized calcium (2, 3). The evidence, in fact, is quite convincing that the "stimulus secretion coupling" mechanism of these secretory cells may be mediated in part by stimulant-induced changes in the cellular influx or efflux rates of calcium. This in turn would result in an alteration in the availability of intracellular calcium to react with a specific cellular-binding protein which may be a rate-limiting step in the secretory process. The role of calcium in the regulation of gastrin release is uncertain. It has been reported that gastrin release is stimulated in certain species by elevations in either luminal or systemic calcium levels (4–6). It, however, is unclear whether these changes in gastrin release are a direct result of an influx of calcium into the G cell, or alternatively may be caused by calcium-induced changes in another gastric factor which in turn influences gastrin release by a paracrine mechanism of action. In order to study this question further we investigated the influence of calcium on gastrin release from an isolated and enriched preparation of G cells.

Materials and Methods. An isolated and enriched preparation of G cells was prepared from rodent antral mucosae by the combined methods of selective pronase digestion followed by unit gravity velocity sedimentation as previously described (1). This resulted in a fraction of cells of which the G cells represented 15–25% of the population, an approximate 50 to 100-fold purification over their number in the intact mucosa.

In the secretory studies, $50-200 \times 10^3$ of the enriched G cells were added to siliconized glass tubes containing 1 ml of normal Krebs–Ringer bicarbonate (KRB) buffer in the presence and absence of varying concentrations of the test agents. In the first series of experiments the cells were incubated under basal conditions for 10 min at 37° in an atmosphere of 95% air/5% CO_2 in one of the following solutions: (i) normal KRB buffer (2.4 m*M* $CaCl_2$); (ii) calcium-free KRB buffer (0.04 m*M* $CaCl_2$), and calcium-free KRB containing 0.2 m*M* EGTA (0.01 m*M* $CaCl_2$). The concentration of $CaCl_2$ in the medium, as indicated above, was determined by atomic absorption spectrometry. An additional set of tubes was incubated in the above three types of media to which the *in vitro* gastrin secretory stimulant, peptone (7), was added to make a final concentration of 2.5%.

In the second series of experiments, en-

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riched G cells were incubated under basal conditions in modified KRB medium containing varying concentrations of $CaCl_2$ (0–5 m*M*). In the final series of experiments the endocrine cells were incubated for 10 min in normal KRB medium (2.4 m*M* $CaCl_2$) containing varying concentrations of Verapamil (0–100 μM) (Knoll Pharmaceutical, Whippany, N.J.).

In all experiments the cells were separated from the medium at the termination of the incubation period by centrifugation (200 *g*, for 10 min at 4°). The medium was then collected, heat extracted at 96° for 5 min, and stored at −20°.

Gastrin concentration in the medium was determined by radioimmunoassay by a technique previously described in detail (8–10). Antisera No. 1296 (kind gift of Dr. J. H. Walsh, Center for Ulcer Research and Education) which specifically recognizes all the known molecular forms of gastrin was used in all assays.

The medium concentrations of both ATP and DNA were monitored in order to compare the leakage of these intracellular markers from cells incubated in medium containing normal or abnormal calcium concentrations. The media was initially extracted with an equal volume of 0.4 *N* PCA and after the appropriate preparative steps (7) the DNA concentration of the precipitate was determined by the method of Giles and Meyers (11) whereas ATP was measured fluorometrically (12).

Results. In the first series of experiments we investigated the influence of the extracellular calcium concentration on both basal and peptone-stimulated gastrin release. The results shown in Fig. 1 demonstrate that basal gastrin release was moderately but significantly increased by incubating the cells in calcium free medium in comparison to hormone release in medium containing a normal concentration of the cation. In contrast, peptone-stimulated gastrin release did not appear to be influenced by the extracellular calcium concentration, as the medium gastrin concentration did not significantly vary among the three groups.

The role of calcium in the regulation of basal gastrin release was further investigated. In the next experiment enriched G cells were incubated in medium containing varying concentrations of $CaCl_2$ from 0 to 5 m*M* under basal conditions. The results shown in Fig. 2 demonstrate that basal gastrin release was significantly elevated in comparison to normal when cells were incubated in medium containing 0 and 0.3 m*M* $CaCl_2$. Basal gastrin release, as reflected by the medium hormone concentration, then decreased as the calcium concentration of the buffer was raised. It should be noted that a significant difference in basal gastrin release was detected between cells incubated in medium containing $CaCl_2$ at a concentration of 0.3 m*M* and those incubated at concentrations of 1.2 m*M* and greater. It in fact was determined that basal gastrin release was significantly reduced in comparison to normal control values (2.4 m*M*) when the cells were incubated in medium containing abnormally high calcium levels (3.8 and 5.0 m*M* $CaCl_2$). This calcium-induced alteration in basal gastrin release most probably is not due to

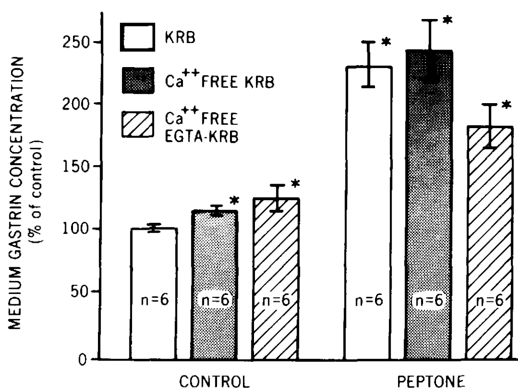

FIG. 1. Basal and peptone-stimulated gastrin release of an isolated and enriched fraction of G cells incubated for 10 min at 37° in (a) normal KRB buffer; (b) calcium-free KRB buffer; and (c) calcium-free KRB buffer containing 0.2 m*M* EGTA. The data in this and all other figures are expressed as a means ±1 SEM of the percentage of the medium gastrin concentration of cells incubated in KRB buffer containing 2.4 m*M* $CaCl_2$ (controls). The mean gastrin concentration released into the medium by cells incubated in KRB buffer in this experiment was 1.70 p*M*/10^6 cells. Asterisks in this and all other figures represent a significant difference in medium hormone concentration ($P < 0.05$) in comparison to incubated control values (Normocalcemic KRB).

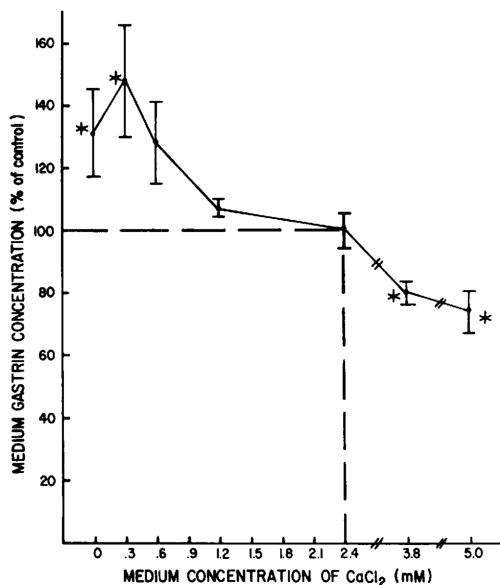

FIG. 2. Alterations in basal gastrin release when isolated enriched G cells are incubated in KRB medium containing varying concentrations of $CaCl_2$. The mean control medium gastrin concentration in this study was 3.78 pmole/10^6 cells.

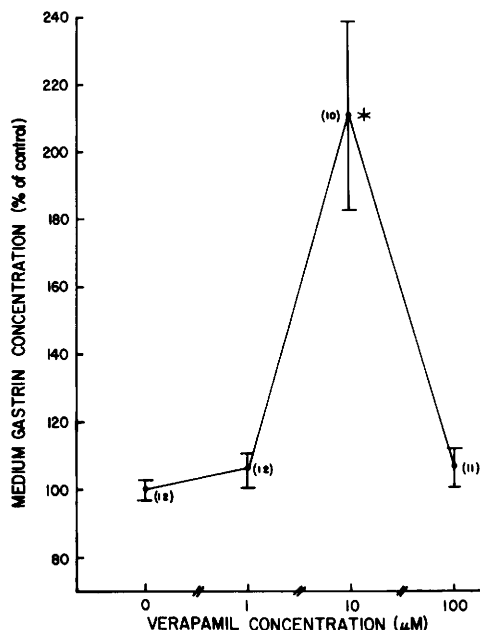

FIG. 3. Alterations in basal gastrin release when isolated enriched G cells are incubated in medium containing varying concentrations of Verapamil. The mean control medium gastrin concentration in this study was 1.51 pmole/10^6 cells. Numbers enclosed within the parentheses represent the numbers of observations for each concentration of the inhibitor.

alteration in cell permeability since we failed to detect a leakage of the intracellular markers, ATP and DNA, into the medium at any of the tested doses of $CaCl_2$.

In the final series of experiments we investigated the alterations in basal gastrin release when calcium influx into the cells was inhibited. This was accomplished by incubating the cells in normal KRB medium (2.4 mM $CaCl_2$) containing varying doses of the calcium channel blocking drug, Verapamil. It has been reported that calcium influx into most isolated cells is maximally inhibited at a concentration of 10 μM, with cytoxic effects being recorded at higher doses of the drug (11). Figure 3 demonstrates that basal gastrin release was markedly and significantly increased when the cells were incubated in medium containing 10 μM Verapamil whereas the other doses of the drug were ineffective stimulants of release. It, however, should be noted that cellular gastrin concentration was significantly reduced below normal values when cells were incubated in medium containing Verapamil at a concentration of 100 μM and greater suggesting that cellular damage may have occurred at these doses. This potential cytotoxic effect of the drug may explain why gastrin secretory stimulation was abolished at the highest dose of Verapamil that was tested.

Discussion. The results presented above indicate that intracellular calcium may have an inhibitory influence on basal gastrin release. This is based on the evidence that incubation of an enriched fraction of G cells in medium containing an abnormally low extracellular calcium concentration stimulated gastrin release into the medium whereas high medium calcium concentration proved to be inhibitory. In support of the above findings, it was demonstrated that blocking calcium entry into G cells resulted in a significant elevation in gastrin release into the medium. Thus, it is possible that the gastrin secretory mechanism is stimulated by reducing calcium influx into the G cell. Alternatively, it can be argued that an accelerated efflux of calcium from the G cell which would occur when the cells

are incubated in low calcium containing medium would stimulate gastrin secretion by inducing the release of calcium from intracellular binding sites (i.e., microsomal or mitochondrial organelles) making it available to mediate hormone secretion. Consistent with this possibility, it has been reported that Verapamil induces an instantaneous efflux of intracellular calcium in addition to blocking entry of the cation (11). Our finding that peptone-stimulated gastrin release was uninfluenced by medium calcium concentration is unexplained but it may be attributable to the fact that proteinaceous agents stimulated gastrin release by altering the cellular calcium permeability. This is supported by our recent finding that gastrin release was enhanced a comparable amount by both Verapamil and peptone and that hormone secretion was not further increased when the two agents were given together. It also should be noted that although most secretory cells show a contrasting dependency on calcium as demonstrated here, there are a number of endocrine and exocrine cells that similarly are stimulated when the extracellular calcium concentration is reduced (12–14).

It is presently unclear why the *in vitro* results reported here conflict with the *in vivo* experiments which demonstrate that gastrin release is stimulated by elevations in extracellular calcium concentration. It, however, should be emphasized that a majority of the *in vivo* studies investigated the influence of gross elevations in luminal calcium concentration (20–80 m*M*) on gastrin secretion which far exceed the small perturbations in cation concentration (0–5 m*M*) tested in our system (3, 5). This, however, cannot be the sole explanation for the discrepancy between *in vivo* and *in vitro* responses to changes in extracellular calcium since relatively small elevations in serum calcium concentration have been reported to stimulate gastrin release in certain species (4).

Although the specific cause of these contrasting gastrin secretory responses to calcium was not indentified in this study, several speculative explanations can be offered. First, it is possible that calcium does not directly stimulate the release of gastrin from the G cell *in vivo*, but instead acts by influencing the release of other factors which secondarily results in gastrin secretory stimulation. The requirements for this putative mediator are: (i) that its release is calcium dependent; and (ii) that it either stimulates or inhibits gastrin release. Thus, there are many potential candidates, including both substances which would be released systemically (endocrine) as well as those which are released locally and have a paracrine influence on gastrin release. Since both endocrine as well as paracrine interactions would be minimized in the isolated endocrine cell preparation due to both a removal of other nongastrin endocrine cells and a disruption of intercellular junctions, it would not be surprising that the influence of calcium in gastrin release would differ in the isolated cell system from the *in vivo* situation.

A second explanation that should be considered is that it is possible that the increase in basal gastrin release when the endocrine cells are incubated in low extracellular calcium may reflect a nonspecific increase in cell permeability or "leakiness" which has been reported to occur when other cell systems are placed in calcium-free medium (15). This possibility, however, seems doubtful since we failed to detect the release of intracellular markers (ATP, DNA) into the medium when the enriched G cells were incubated in medium containing low concentrations of $CaCl_2$.

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