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INVOLVEMENT OF Ca^{2+} INFLUX IN F^--STIMULATED PEPSINOGEN RELEASE FROM GUINEA PIG GASTRIC CHIEF CELLS

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Summary: In isolated guinea pig gastric chief cells, pepsinogen release was stimulated by NaF in a dose-dependent manner. Cholecystokinin (CCK) and Ca^{2+}ionophore A23187 had no additional effect on NaF-stimulated pepsinogen release. CCK caused a rapid increase in intracellular free Ca^{2+} concentration ($[Ca^{2+}]i$) monitored by Quin-2 and markedly stimulated inositol phosphate accumulation in chief cells. By contrast, NaF did not cause any change in $[Ca^{2+}]i$. NaF, even at a maximal concentration for pepsinogen release, appeared to be relatively ineffective on inositol phosphate accumulation. On the other hand, NaF markedly stimulated Ca^{2+} influx into chief cells. These results suggest that F^- stimulates pepsinogen release probably by increasing Ca^{2+} influx into chief cells. Since F^- is a well known activator of guanine nucleotide regulatory proteins (G proteins), it is proposed that there may exist a G protein regulating the opening of Ca^{2+} channel in gastric chief cells.

We have recently shown that CCK and carbamylcholine stimulate pepsinogen release from isolated gastric chief cells by increasing $[Ca^{2+}]i$, measured with Quin-2 (1). Early studies have shown that these pancreatic secretagogues stimulate phospholipase C in pancreatic acinar cells to hydrolyze polyphosphoinositide into 1,2-diacylglycerol and IP_3 (2,3), which has been well known to cause a rapid release of Ca^{2+} from an internal store (2). Intracellular Ca^{2+} mobilization and protein kinase C activated by 1,2-diacylglycerol have been shown to stimulate amylase secretion synergistically (3). Furthermore, it has been recently proposed that G proteins analogous to those linked to adenylate cyclase couple these Ca^{2+}-mobilizing hormone receptors to the activation

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Abbreviations used are: CCK, cholecystokinin; CCK8, COOH-terminal octapeptide of CCK; IP_3, inostol 1,4,5-trisphosphate; G protein, guanine nucleotide regulatory protein; $[Ca^{2+}]i$, intracellular free Ca^{2+} concentration.

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of phospholipase C in pancreatic acinar cells (4), as shown in a variety of other cells (2). It can be speculated, therefore, that there exists a similar intracellular mechanism underlying pepsinogen secretion evoked by CCK and cholinergic agents in gastric chief cells.

Recently, F^- has been shown to mimic the effects of Ca^{2+}-mobilizing hormones through the activation of polyphosphoinositide breakdown and a subsequent increase in $[Ca^{2+}]i$ in isolated hepatocytes (5), neutrophils (6), and platelets (7). Since F^- is well known to be a potent activator of G proteins (8), authors have suggested the involvement of a G protein in the coupling of the receptors for Ca^{2+}-mobilizing hormones to the hydrolysis of polyphosphoinositide in these cells. Thus, F^- appears to be a useful probe for examining whether a G protein actually functions as a transducer between Ca^{2+} mobilizing hormone receptors and the activation of phospholipase C in intact cells. In this study, therefore, to clarify the role of G protein as a signal transducer in pepsinogen secretion stimulated by Ca^{2+}-mobilizing hormones, the effects of F^- on isolated guinea pig gastric chief cells were examined.

We show here that in chief cells F^- also stimulates pepsinogen release probably via intracellular Ca^{2+} mobilization. However, the signal transduction pathway activated by F^- that leads to Ca^{2+} mobilization appears to be distinct from that activated by CCK.

MATERIALS AND METHODS

Isolation and Incubation of Gastric Chief Cells

Isolated gastric chief cells were prepared as previously described (1). For experiments, cells were suspended and incubated at 37°C in an oxygenated medium consisting of 132 mM NaCl, 4.7 mM KCl, 5 mM Na_2HPO_4, 1 mM NaH_2PO_4, 1.1 mM $MgCl_2$, 1.28 mM $CaCl_2$, 0.5% BSA and 0.01% soybean trypsin inhibitor.

Release of Pepsinogen

Pepsinogen release from cells was determined as previously reported (1).

Determination of $[Ca^{2+}]i$

Cells were loaded with Quin-2 by incubating them with 4 μM Quin-2/AM for 20 min at 37°C. Fluorescence measurements were carried out essentially as previously reported (1). Excitation and emission wavelengths were set at 339 nm and 492 nm, respectively.

Measurement of cAMP production in chief cells

2 ml aliquots of cell suspension were incubated with agents in the presence of 0.1 mM isobutylmethyl xanthine for 30 min at 37°C. After centrifugation, intracellular cAMP was extracted and measured by sensitive RIA procedure as described previously (9).

Assay for Inositol Phosphate Formation

Cells were prelabeled with 25 μCi/ml myo-[2-^{3}H]inositol for 2 h at 37°C. Thereafter, cells were washed twice with incubation buffer, and the incubations of cells with agents were carried out for 3 min at 37°C in 500 μl of the incubation buffer containing 10 mM LiCl. After the reaction was terminated by the addition of 1.88 ml of chloroform/methanol/HCl, each aqueous product was processed by the methods as described by Berridge (10) for the analysis of inositol phosphates.

Measurement of $^{45}Ca^{2+}$ Influx

Ca^{2+} influx was measured according to the method of Mauger et al. (11). Briefly, incubation was started by the addition of 3 ml of cell suspension to trace amounts of $^{45}CaCl_2$ (2 μCi/ml) and to test agents. After cells were incubated for 30, 90, 150, or 210 s at 37°C, 500 μl aliquots were taken, centrifuged and washed once with 1 ml of fresh incubation buffer. The radioactivity associated with cells was counted. The Ca^{2+} uptake was liner within the range of these time points (data not shown). The Ca^{2+} influx rate was calculated by using a slope of the linear regression line of Ca^{2+} uptake.

Chemicals

CCK8 was a gift from Squibb Institute Inc. Secretin was obtained from Peptide Institute Inc., Quin-2/AM from Dojin Chemicals, $^{45}CaCl_2$ and myo-[2-^{3}H]inositol from Amersham Co., NaF from Nakarai Chemicals, and Ca^{2+}ionophore A23187 and isobutylmethyl xanthine from Sigma Chemicals.

RESULTS

When gastric chief cells were incubated with increasing concentrations of NaF, a dose-dependent stimulation of pepsinogen release was observed, with a detectable and a maximal stimulation obtained at 3 mM and 20 mM NaF, respectively (Fig. 1). In order to explore the mode of F^- action on pepsinogen release, the effects of secretagogues in combination with NaF on pepsinogen release were tested. When combined with 10 mM NaF, various doses of either CCK8 or Ca^{2+}ionophore, whose action has been shown to be mediated by intracellular Ca^{2+} mobilization (1,12), had no additional effect on pepsinogen release (Fig. 2A,B). By contrast, pepsinogen release in response to various doses of secretin, a secretagogue via cellular cAMP (13), plus 10 mM NaF was almost equal to the calculated additive values (Fig. 2C). Additionally, NaF at concentrations of up to 20 mM showed no significant change in cellular cAMP content. A detectable increase in cellular cAMP level occurred at 30 mM NaF, which increased cAMP content from 0.76±0.06 pmol/mg of protein (basal) to 1.06±0.04 pmol/mg of protein (mean±S.E., n=4). These results indicate that NaF-stimulated pepsinogen release may involve cellular Ca^{2+} mobilization.

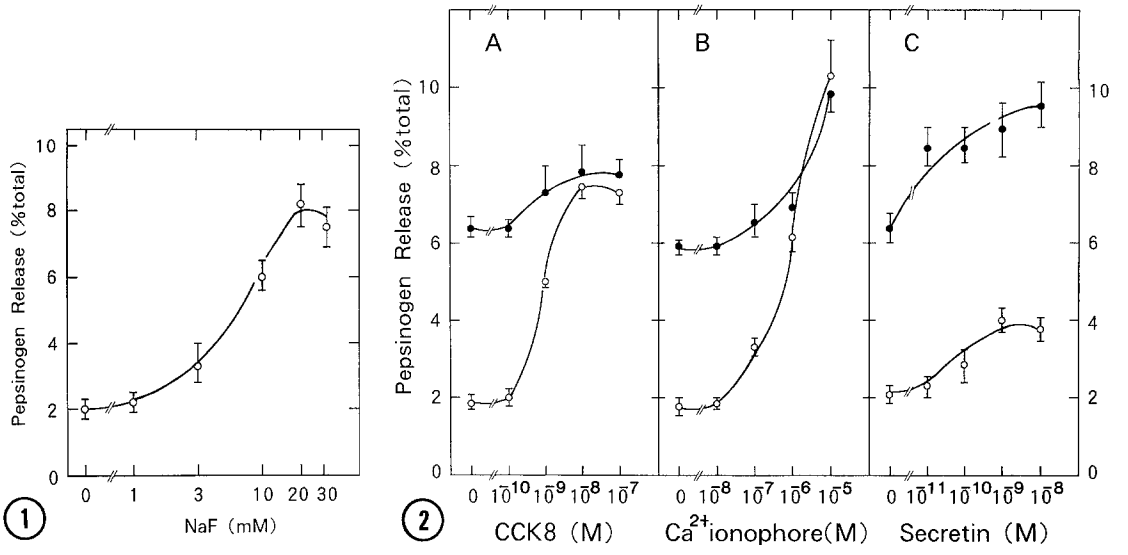

Fig. 1. Effects of various concentrations of NaF on pepsinogen release. Gastric chief cells were incubated at 37°C for 30 min with increasing concentrations of NaF. Each value shown is the mean ± S.E. of triplicate determinations from a representative of three separate experiments.

Fig. 2. The combined effects of NaF and various secretagogues on pepsinogen release. Gastric chief cells were incubated with increasing concentrations of various secretagogues in the presence (●) or absence (o) of 10 mM NaF. Values shown are the mean ± S.E. of triplicate determinations from a representative of three separate experiments.

Accordingly, we next examined whether F^- actually causes an increase in $[Ca^{2+}]i$ and stimulates inositol phosphate generation in chief cells. As previously reported (1), 10^{-8}M CCK8 caused a rapid increase in $[Ca^{2+}]i$; however 20 mM NaF did not affect $[Ca^{2+}]i$ measured by Quin-2 (Fig. 3A). Moreover, following the addition of 20 mM NaF, 10^{-8}M CCK8 was able to stimulate an increase in Quin-2 fluorescence (Fig. 3B). When ^{3}H-insoitol labeled chief cells were incubated with varying doses of NaF, a detectable stimulation of inositol phosphate formation was obtained at 10 mM NaF (Fig. 4). In addition, 30 mM NaF, a supramaximal concentration for pepsinogen release, stimulated a 1.5-fold increase of control value, whereas 10^{-8}M CCK8 stimulated a 6-fold increase of control value (Fig. 4).

Since the mechanism leading to Ca^{2+} mobilization activated by F^- seems to be different from that activated by CCK, we next tried to determine whether F^- stimulates Ca^{2+} influx into cells. The basal Ca^{2+} influx calculated was 621±63 pmol/min/mg protein (mean±S.E., n=4). When the effects of increasing concentrations of NaF on Ca^{2+} influx into chief cells was tested, NaF stimulated

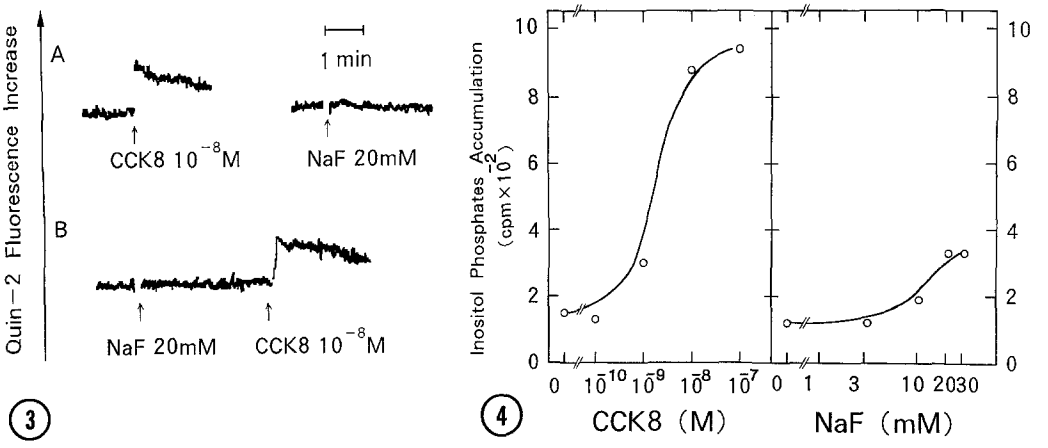

Fig. 3. Representative recordings showing effects of NaF and CCK8 on Quin-2 fluorescence in chief cells. CCK8 was added to the medium 3 min after the addition of NaF (B).

Fig. 4. Effects of CCK8 and NaF on inositol phosphate generation in gastric chief cells. ^{3}H-inositol labeled chief cells were incubated at 37°C for 3 min with increasing concentrations of CCK8 or NaF. Each value is expressed as the radioactivity of inositol bisphosphate plus IP_3 formed during incubation and the mean of triplicate determinations from a representative of three separate experiments.

the initial Ca^{2+} influx rate in a dose-dependent manner, with a detectable stimulation observed at 3 mM NaF (Fig. 5). The minimum effective dose of NaF necessary for an increase in Ca^{2+} influx was the same as that for pepsinogen release.

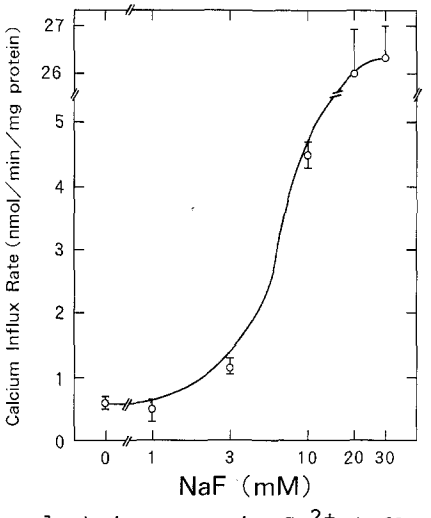

Fig. 5. A dose-dependent increase in Ca^{2+} influx into chief cells by NaF. $^{45}Ca^{2+}$ and various doses of NaF were added at time 0, and Ca^{2+} uptake was measured at 30, 90, 150, and 210 s as described under "Methods". Values shown are the mean ± S.E. from four separate experiments.

DISCUSSION

The major finding of the present study is that F^-, which has been shown to activate a certain type of G proteins (8) thereby mimicking the actions of many hormones (5,6,7), also stimulates pepsinogen release from gastric chief cells. Several lines of evidences suggest that this stimulatory effect of F^- may be due to F^--induced cellular Ca^{2+} mobilization. First, NaF stimulated pepsinogen release in a dose-dependent manner and had a similar efficacy on pepsinogen release from chief cells when compared with that of CCK. In combination with NaF, CCK or Ca^{2+}ionophore, a secretagogue via cellular Ca^{2+} mobilization (1,12), did not cause a further increase in pepsinogen release, whereas secretin, a cAMP dependent secretagogue (12,13), showed an additional effect on pepsinogen release stimulated by NaF. In chief cells (12), it has been previously recognized that potentiation of pepsinogen release occurs with a secretagogue that increases cAMP, plus a secretagogue which stimulates Ca^{2+} mobilization, but does not occurs with two secretagogues, each of which has the same mode of action, as shown in pancreatic acinar cells (13). Second, although F^- is known to be a potent activator of both stimulatory and inhibitory G proteins (8), which are described as regulating adenylate cyclase activity, NaF at submaximal and maximal concentrations stimulating pepsinogen release did not affect cellular cAMP levels in chief cells. Third, of interest is the observation that NaF facilitated Ca^{2+} influx into chief cells. The minimum effective dose of NaF necessary for stimulation of Ca^{2+} influx is found to correspond well to that for pepsinogen release. Therefore, these results suggest that the stimulatory action of NaF on pepsinogen release may involve cellular Ca^{2+} mobilization but not the cAMP-dependent pathway.

However, in chief cells the mechanism activated by F^- to cause Ca^{2+} mobilization is considered to be distinguishable from that of a Ca^{2+}-dependent secretagogue such as CCK, according to the following observations. In contrast to the rapid increase in $[Ca^{2+}]i$ observed with CCK, NaF didn't affect $[Ca^{2+}]i$ monitored by Quin-2. Furthermore, CCK caused an increase in $[Ca^{2+}]i$ even in the presence of priorly added NaF, suggesting that CCK- and NaF-induced Ca^{2+} mobilization may be derived from different Ca^{2+} sources (14). In pancreatic acinar cells, CCK has been shown to simulate the breakdown of polyphosphoinositide to produce IP_3 (2), which thereby causes intracellular Ca^{2+} mobilization from an

internal Ca^{2+} store (2). In chief cells, we have demonstrated that CCK markedly stimulates inositol phosphate accumulation, indicating that CCK also causes intracellular Ca^{2+} mobilization by stimulating hydrolysis of polyphosphoinositide in chief cells. On the other hand, NaF at relatively higher concentrations for pepsinogen release stimulated inositol phosphate generation and these effects were rather weak as compared with those of CCK. Hence, although we cannot totally exclude a remote possibility that F^- action may involve IP_3 generated by polyphosphoinositide hydrolysis, the present data propose another possibility that NaF may stimulate pepsinogen release by increasing Ca^{2+} influx into chief cells.

Although NaF stimulated Ca^{2+} influx in the present study, any change in $[Ca^{2+}]i$ in response to NaF was not detected by Quin-2. The reason for the inability of Quin-2 to detect the NaF signal is not fully understood at present. However, the Ca^{2+} chelating activity of Quin-2 may account for the inability to detect the NaF action on $[Ca^{2+}]i$ monitored by Quin-2. A similar observation that ACTH-induced Ca^{2+} influx is detected by aequorin but not by Quin-2 in adrenal glomerulosa cells has been also reported (15).

In addition to the originally described role of G proteins in the adenylate cyclase system, G proteins have been recently found to mediate many other cellular processes (8). Most recently, it has been reported that a G protein directly regulates mammalian cardiac Ca^{2+} channels (16). The present finding that F^- stimulates Ca^{2+} influx also suggests the possible involvement of a G protein in regulating the opening of Ca^{2+} channel in gastric chief cells. This is because F^- is well known to be a potent activator of G proteins.

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