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CHOLECYSTOKININ ELEVATES CYTOSOLIC CALCIUM IN SMALL CELL LUNG CANCER CELLS

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The ability of cholecystokinin (CCK) to elevate intracellular Ca^{2+} levels in small cell lung cancer cells was investigated using the fluorescent Ca^{2+} indicator Fura 2. CCK-8 elevated the cytosolic Ca^{2+} levels in cell line NCI-H345 in a dose dependent manner. Nanomolar concentration of CCK-8 elevated cytosolic Ca^{2+} levels in the absence or presence of extracellular Ca^{2+}. Potent CCK agonists such as gastrin-1 and nonsulfated CCK-8 but not inactive compounds such as CCK-27-32-NH_2 elevated cytosolic Ca^{2+} levels. These data suggest that CCK receptors may regulate the release of Ca^{2+} from intracellular organelles in small cell lung cancer cells.

Cholecystokinin (CCK) is a peptide biologically active in the CNS and periphery (1). In the brain, CCK is colocalized with dopamine in some CNS neurons (2). When released it may interact with high affinity binding sites termed CCK-B receptors (3-5). CCK alters rodent behavior and may function as a brain neuromodulator (6). In the periphery, CCK is localized to intrinsic neurons of the myenteric plexus (7). When released, CCK may bind to CCK-A receptors in the stomach, pancreatic acini and other organs (8-10). In pancreatic acini, CCK stimulates phosphatidylinositol turnover, ^{45}Ca efflux, cGMP levels and amylase secretion (8,11). In the periphery, CCK is a potent satiety agent (12). These data indicate that CCK is biologically active in certain CNS and peripheral cells.

Peptides may also be active in malignant cells. Bombesin (BN)-like peptides are synthesized in (13) and secreted from small cell lung cancer SCLC cells (14). Because BN binds to cell surface receptors (15), elevates

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cytosolic Ca^{2+} (16) and stimulates growth (17), it may function as an autocrine growth factor. Recently, high affinity CCK-8 binding sites were identified on SCLC cells (18). Here we investigated if CCK elevates cytosolic Ca^{2+} in SCLC cells.

MATERIALS AND METHODS

The human tumor cell line NCI-H345 was cultured in serum-supplemented medium (RPMI 1640 containing 10% heat inactivated fetal calf serum) in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. One day after a medium change the cells were harvested by centrifugation at 1,000 x g for 10 minutes. Cells were washed and resuspended in SIT medium (RPMI 1640 containing 3 x 10^{-8} M Na_2SeO_3, 5 μg/ml bovine insulin, and 10 μg/ml human transferrin (Sigma Chemical Co., St Louis, MO)).

SCLC cells (2.5 x 10^6/ml) were resuspended in SIT medium which contained 20 mM HEPES/NaOH (pH 7.4) for loading of the fluorescent Ca^{2+} indicator Fura 2 AM (19). Cell suspensions (10 ml) were incubated with 5 μM Fura 2 (Calbiochem Inc., La Jolla,CA) at 37°C for 30 minutes in a shaking water bath. Cells were centrifuged at 150 x g for 2 minutes, and the pellet containing SCLC cells loaded with Fura 2 was resuspended (2.5 x 10^6 cells/ml). The loaded cells were transferred to a spectrofluorometer equipped with a magnetic stirring mechanism and a temperature (37°C) regulated cuvette holder. Fluorescence was monitored at excitation and emission wavelengths of 340 nm and 510 nm respectively. Cytosolic Ca^{2+} concentrations were calculated employing methods similar to those used previously (20) with correction for the presence of extracellular Fura 2. Synthetic CCK analogues were obtained from Peninsula Laboratories, San Carlos, CA.

RESULTS

The basal cytosolic free Ca^{2+} concentration was 150 nM _+ 20 nM. Previously, we found that BN-like peptides transiently elevated the cytosolic Ca^{2+} levels to 190 nM. Here CCK-8 elevated cytosolic Ca^{2+} in a dose dependent manner. Figure 1A shows that 0.1 nM CCK-8 did not alter the cytosolic Ca^{2+} levels. Within 15 seconds after addition of 1 nM CCK-8 the cytosolic Ca^{2+} levels weakly increased (Fig. 1B). Using 10, 100 or 1000 nM CCK-8, the cytosolic Ca^{2+} strongly increased from 150 to 180 nM, followed by a rapid decline (Fig. 1C-E). These data indicated that CCK-8 transiently increases the cytosolic Ca^{2+} levels.

The source of the CCK-induced increase in cytosolic Ca^{2+} was investigated. CCK-8 caused a strong Ca^{2+} response (Fig. 2A). When 5 mM EGTA was added there was a decrease in the relative fluorescence due to removal of Ca^{2+} that was bound to extracellular Fura-2. Nonetheless, when CCK-8 was added there was a significant increase in the cytosolic Ca^{2+} almost identical

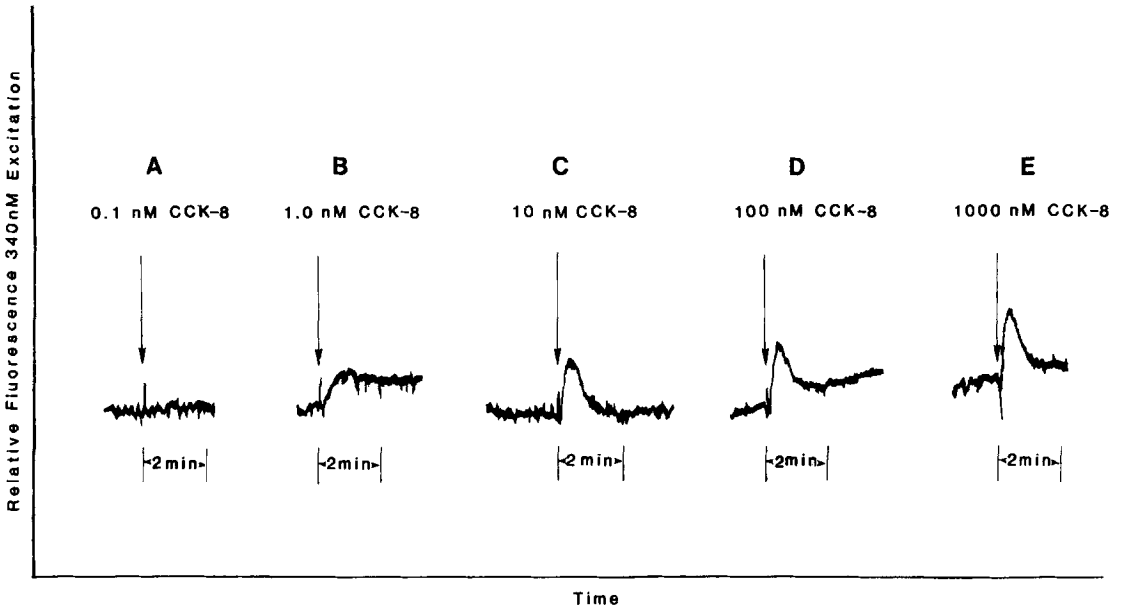

Figure 1. Dose response curve of CCK-8. The ability of (A) 0.1 nM CCK-8, (B) 1 nM CCK-8, (C) 10 nM CCK-8 (D) 100 nM CCK-8 and (E) 1000 nM CCK-8 to elevate cytosolic Ca^{2+} in SCLC cell line NCI-H345 was determined using Fura 2. This figure is representative of 3 other determinations.

to that obtained in the presence of extracellular Ca^{2+}. These data indicate that CCK-8 causes release of Ca^{2+} from intracellular pools.

The ability of other CCK analogues to elevate cytosolic Ca^{2+} was investigated. At a 10 nM dose, CCK-8, gastrin-1, nonsulfated CCK-8 and CCK-4 elevated the cytosolic Ca^{2+} whereas CCK-27-32-NH_2 did not (Table I). These data indicate that CCK-8, gastrin-1, nonsulfated CCK-8 and CCK-4 may function as CCK receptor agonists whereas CCK-27-32-NH2 is inactive. Peptides

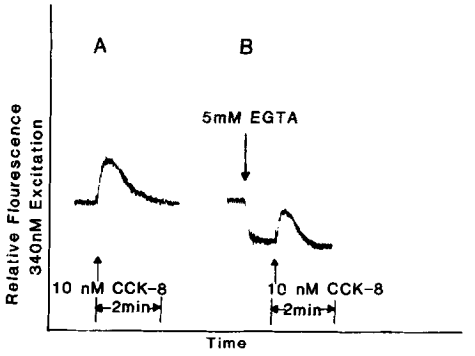

Figure 2. Ca^{2+} response to CCK-8 in the presence and absence of extracellular free Ca^{2+}. Fura 2 measurements were made in the presence of 0.4 mM $CaCl_2$ in the absence (A) and presence (B) of 5 mM EGTA. CCK-8 was added at a concentration of 10 nM.

Table I. Ability of CCK analogues to elevate cytosolic Ca^{2+}

Peptide	Ca^{2+} response
CCK-8	+
gastrin-1	+
nonsulfated CCK-8	+
CCK-4	+/-
CCK-27-32-NH_2	-

The ability of various CCK agonists (10 nM) to elevate cytosolic Ca2+ is indicated. (+), Strong response; (+/-), weak response; (-) inactive. The structure of the gastrin I and CCK is shown below:

Gastrin I pGlu-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2

CCK-8 Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2
SO_3

unrelated structurally to CCK such as somatostatin and vasoactive intestinal polypeptide had no effect on the cytosolic Ca^{2+} (16).

CCK-transiently increased the cytosolic Ca^{2+} followed by a rapid decline. The decline may be due to receptor desensitization rather than depletion of intracellular Ca^{2+} pools. Initially, 10 nM CCK-8 caused a strong Ca^{2+} response. When 10 nM BN was subsequently added there was a strong Ca^{2+} response (Fig. 3A). Similarly, addition of 10 nM neurotensin after addition of 10 nM CCK-8 caused a strong Ca^{2+} response (Fig. 3B). In contrast, addition

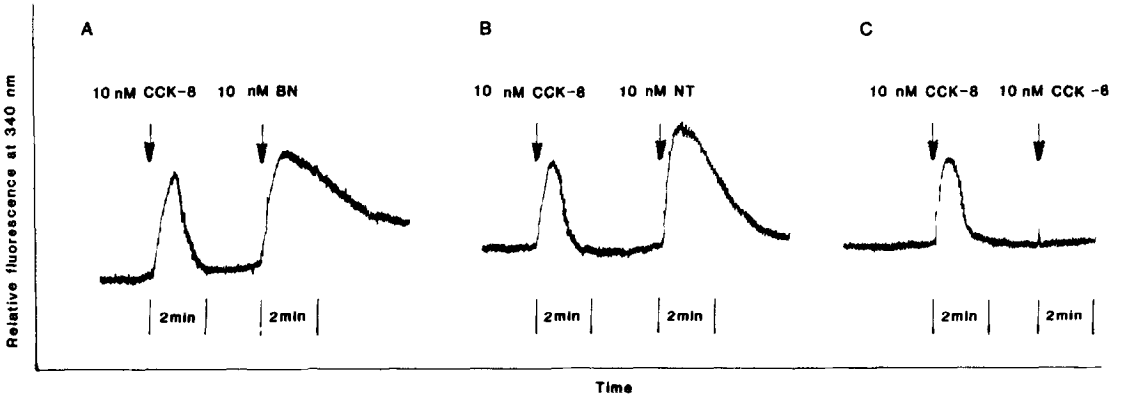

Figure 3. Ca^{2+} response in the presence of various peptides. Fura 2 measurements were made after addition of 10 nM CCK-8 followed by 10 nM BN (A), 10 nM CCK-8 followed by 10 nM neurotensin (B) and 10 nM CCK-8 followed by 10 nM CCK-8 (C).

of 10 nM CCK-8 after initial addition of 10 nM CCK-8 resulted in no response (Fig. 3C). These data indicate that CCK, neurotensin and BN elevate cytosolic Ca^{2+} through distinct neuropeptide receptors.

DISCUSSION

Previously, we characterized receptors for CCK in SCLC cells (18). ^{125}I-CCK-8 bound with high affinity (Kd = 2.4 nM) to a single class of sites (Bmax = 1700/cell). CCK-8, gastrin-1, CCK-33, nonsulfated CCK-8 and CCK-4 inhibited specific ^{125}I-CCK-8 binding with IC_{50} values of 0.9, 1.4, 4.5, 6.5 and 25 nM respectively. Because CCK-8 bound with slightly higher affinity than did gastrin-1, CCK-33 or nonsulfated CCK-8, SCLC CCK receptors may be of the CNS or CCK-B type. Here we investigated the ability of CCK agonists to elevate cytosolic Ca^{2+} in SCLC cells.

CCK-8 elevated cytosolic Ca^{2+} in a dose dependent manner. The ED_{50} for CCK-8 was approximately 1 nM which closely agrees with the IC_{50} obtained from the binding data. Previously, it was determined that CCK-8 stimulated phosphatidylinositol turnover (11). The inositol-1,4,5 trisphosphate released may cause release of Ca^{2+} from intracellular pools. Because the chelation of extracellular Ca2+ by the addition of EGTA had no effect on the CCK-8 induced Ca^{2+} response, CCK-8 may cause release of Ca^{2+} from intracellular stores and not plasmalemmal Ca^{2+} influx.

In addition to CCK-8, 10 nM gastrin-1 and nonsulfated CCK-8 caused a strong Ca^{2+} response. CCK-4 (10 nM) caused a weak Ca^{2+} response whereas CCK-27-32-NH_2 (10 nM) was inactive. Preliminary data (J. Staley, unpublished) indicate that 10 nM L-364,718 or 10 nM L-365,260 had no effect on the basal Ca^{2+} levels, but that 10 nM L-365,260 antagonized the ability of 10 nM CCK-8 to elevate cytosolic Ca^{2+} levels. Previously, the benzodiazepine analogues L-364,718 and L-365,260 were demonstrated to be potent CCK-A and CCK-B receptor antagonists (21,22).

Besides CCK, BN and neurotensin release Ca^{2+} from intracellular pools using cell line NCI-H345 (13,23). BN and neurotensin stimulate the clonal growth of SCLC <u>in vitro</u> (17,24). Currently, we are investigating if CCK

alters the growth of SCLC cells. In summary, the demonstration that CCK elevates the cytosolic Ca^{2+} indicates that SCLC CCK-B receptors are biologically active.

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