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In Vitro Secretion of Peptides of the Calcitonin Family: Calcitonin, Katacalcin, and Calcitonin Gene-Related Peptide

Friedhelm Raue,* Hubert Serve, Eckard Rix, and Reinhard Ziegler

A tissue culture explant and a primary cell culture of malignant C-cells have been established to study the secretion pattern of calcitonin (CT), katacalcin (KC), and CT gene-related peptide (CGRP) in vitro under different conditions. Within the first five days of culture the spontaneous secretion of the peptides in both systems dropped to and remained at a constant level. With increasing calcium (Ca) concentration in the medium, a dose dependent release of CT, KC and, in cell culture, of CGRP was observed. BAY-K-8644, an analog of the Ca channel blocker nifedipine, enhanced the CT secretion to the same extent as Ca in a concentration of 10^{-4}M in tissue, and of 10^{-3}M in cell culture. This stimulation effect of BAY-K-8644 could be inhibited by an equimolar amount of nifedipine. Medullary thyroid carcinoma tissue explants as well as primary cell culture are therefore reliable models for studying the secretion pattern of peptides of the CT family. The effect of BAY-K-8644 and nifedipine suggests a physiological role of Ca channels in stimulus-secretion coupling. (Henry Ford Hosp Med J 1987;35:143-6)

The secretion pattern of peptides of the calcitonin (CT) family—CT, katacalcin (KC), and CT gene-related peptide (CGRP)—has been studied in vitro under different conditions. As calcium (Ca) appears to be the major physiological regulator of hormone release, the role of Ca channels in stimulus-secretion coupling has been elucidated by using the Ca channel blocker nifedipine and the recently discovered Ca channel activator BAY-K-8644 (1,2). These substances were tested in tissue culture explants of medullary thyroid carcinoma (MTC) cell cultures. Histological and immunohistological studies were performed.

Materials and Methods

Organ cultures
Sterile pieces of MTC tissue removed at surgery were divided into 1 to 2 mm pieces and three were placed in organ culture dishes as described recently (3). The medium was changed every day and assayed for CT, KC, and CGRP. On days five to ten of culture period, stimulation tests were done. At different time intervals and at the end of the experiment, tissue cultures were fixed for immunocytochemical and electron-microscopic (EM) studies.

Cell cultures
Sterile pieces of MTC tissue were washed in Ca^{2+} and Mg^{2+} free phosphate-buffer solution (PBS) (Seromed, Munich, West Germany), and minced into small pieces. Aliquots of 10^6 viable cells per mL were further incubated in RPMI 1640 (Seromed, Munich, West Germany) as described recently (4). The medium was changed every two to six days and assayed for CT, KC, and CGRP. For stimulation tests cells were detached from the flask surface by rinsing them gently with Ca^{2+} and Mg^{2+}-free PBS. After 53 days of cultivation, cells were prepared for EM studies.

Stimulation tests
At various times between the three to 14 days of cell and tissue culture, stimulation tests with different substances were performed. Tissue: Ca^{2+} 0.8, 2.5, 5, 10, 20 mM; BAY-K-8644 (Bayer AG, Leverkusen, West Germany) 10^{-5} M, 10^{-4} M; nifedipine (Bayer AG, Leverkusen, West Germany) 10^{-3} M. Cells: Ca^{2+} 0.8, 2.5, 5 mM; BAY-K-8644 10^{-3} to 10^{-2} M; nifedipine 10^{-3} M. Drugs were directly dissolved in the culture medium. Five tissue cultures were incubated in fresh medium for two hours (pre-stimulation period used as control), the medium was removed and replaced by fresh medium with different substances and again incubated for two hours (stimulation period). Peptide concentrations were measured and changes were calculated as percentage of the respective control. The significance of differences between means was calculated using a two-tailed t-test.

Radioimmunoassay
Human CT was determined by a radioimmunoassay (5). Detection limit was 0.05 ng/mL, intra-assay variation 4.9%, and inter-assay variation 12.4%. Katacalcin radioimmunoassay was established using an antiserum against synthetic KC (6,7). Detection limit was 0.1 ng/mL, intra-assay variation 5.2%, and interassay 11.9%. CGRP was measured by radioimmunoassay with reagents purchased from Peninsula Laboratories (Merseyside, England) and Cambridge Research Biochemicals (Harston, England). Detection limit was 0.6 ng/mL, intra-assay variation 6.4%, and interassay 14.4%.

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Fig 1—Spontaneous release of CT, KC, CEA, and CGRP into culture medium from MTC tissue culture explants. Each point is the mean ± SD of 20 separate culture dishes (ng peptide/mL medium/24 hours).

Fig 3—Effect of BAY-K-8644 (BAY), nifedipine (NIF), and Ca, alone or in combination, on release of CT from MTC tissue explants. Bars represent the mean ± SD (n = 5).

Results

Tissue culture

The spontaneous release of CT, KC, and CGRP into the medium showed a decrease within the first five days, then stabilized. CT and KC levels remained mostly constant over the observation period (80 days), whereas CGRP became undetectable (Fig 1). After addition of Ca to the medium on day seven of culture, a significant dose-related increase in the release of CT and KC was observed (Fig 2). Addition of 10⁻⁴ M BAY-K-8644 to the culture medium produced a similar increase in the amount of CT released as 5 mM Ca (Fig 3). This stimulatory effect of BAY-K-8644 could be completely inhibited by an equimolar amount of nifedipine (Fig 3). Viable human MTC tissue could be examined morphologically in vitro over a period of at least 50 days. Nearly all cells showed a positive immunocytochemical reaction for CT, KC, and CGRP (Fig 4).

Cell culture

Concentrations of CT, KC, and CGRP in the MTC cell culture medium also dropped within the first five days (Fig 5). Dose response effects of Ca on CT, KC, and CGRP secretion are shown in Fig 6. BAY-K-8644 caused a significant dose-dependent stimulation of CT and KC, while an equimolar concentration of nifedipine in the presence of BAY-K-8644 inhibited this stimulation completely (Fig 7). Semithin and ultrathin sections of the C-cell carcinoma cultures showed a cluster-like growth of the tumor cells with intercellular spaces forming the so-called pseudofollicular spaces. Nearly all cells contained different amounts of secretory granules which have the typical electron-dense appearance of the CT-containing granules.

Discussion

Tissue explants as well as primary cultures of human MTC were cultivated in vitro over a period of ten to 50 days while continuously secreting peptides of the CT family. A parallel release
Fig 4—Distribution of CT (A), KC (B), and CGRP (C) immunoreactivity in paraffin sections of explants of a medullary carcinoma at day five of culture period. The antibody dilutions were 1/1000 for the CT antibodies and 1/500 for KC and CGRP antibodies. (Magnification A: ×120, B and C: ×220.)

of KC and CT in vitro occurred under basal as well as under stimulation conditions. This concomitant secretion has also been described in perfused human MTC (8). CT and KC, the N-terminal peptide of pro-CT, are derived from the same precursor. An equimolar ratio of CT/KC secretion would be expected and could be confirmed in vivo (6,7) and in vitro (9). CGRP secretion dropped rapidly in the first days of tissue culture and CGRP concentration in the medium was below the detection limit. However, all three peptides could be demonstrated by immunoperoxidase staining at the end of the culture period. CGRP secretion might be regulated differently.

MTC tissue explants as well as primary cell cultures are useful models for studying the secretion pattern of peptides of the CT family which might reflect the expression of CT gene in MTC. BAY-K-8644 produces biological effects similar to those seen with increasing extracellular Ca concentration in MTC tissue explants and in C-cell carcinoma cell culture. This enhancement of peptide secretion by BAY-K-8644 and Ca is completely inhibited by the Ca channel blocker nifedipine. BAY-K-8644 shows a competitive antagonism with nifedipine and seems to act as a Ca channel activator, suggesting a physi-

Fig 5—Spontaneous release of CT, KC, and CGRP into culture medium from MTC cell culture. Each point is the mean ± SD of five separate culture dishes (mg peptide/mL medium/24 hours). *p < 0.01 vs control.
Fig 6—Influence of increasing amounts of Ca in the medium on CT, KC, and CGRP secretion from MTC cell culture. *p < 0.01 vs control.

The physiological role of Ca channels in hormone secretion. Similar effects of BAY-K-8644 have been described for a rat C-cell line (1) and rat thyroid explants (2). BAY-K-8644 is a useful substance to investigate the cellular mechanisms involved in Ca-dependent hormone release.

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Fig 7—Effect of BAY, NIF, and Ca alone or in combination on release of CT and KC from MTC cell culture. Bars represent the mean ± SD (n = 5). *p < 0.01 vs control.

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