Neuropeptide stimulation of calcium flux in human lung cancer cells: Delineation of alternative pathways

(calcium ion dynamics/cellular differentiation/growth agonists/cyclic AMP)

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Contributed by Theodore T. Puck, December 27, 1989

ABSTRACT Calcium ion flux following the administration of a series of neuropeptides, N^6, O^2 -dibutyryl adenosine 3',5'-cyclic monophosphate, and serum was monitored by flow cytometry in selected lung and breast cancer cell lines and Chinese hamster ovary cell line CHO-K1. Calcium ion flux was monitored in individual cells by flow cytometry using the indicator indo-1 AM. Five groups of neuropeptides produced calcium flux changes in lung cancer cell lines and CHO-K1 cells but not in breast cancer cells. The peak increase in free calcium was reached within 10 sec of peptide administration and declined to resting levels in 70–120 sec. When two or more members of the same group were administered simultaneously, calcium flux changes were identical to that produced by each single peptide. When two or more members of different groups were administered simultaneously, an increased calcium release occurred. When identical peptides or peptides from the same group were administered sequentially after the return of calcium concentrations to resting values, no calcium flux resulted from the second peptide. When peptides from different active groups were administered sequentially, a new calcium flux occurred after each peptide. These data are interpreted to mean that members of each active group of peptides trigger a different calcium flux pathway. Thus, many such pathways and different metabolic states exist within the cell. Elucidation of calcium flux pathways in normal and cancer cells may lead to greater understanding of the nature of the malignant defect.

A transient increase in the cellular concentration of free calcium ions as a result of addition of a variety of hormones, growth factors, and other agents has been documented in many cell systems (1–5). The nature and biological function of such calcium release have been enigmatic. It has been proposed that cytosolic calcium acts as an oscillator whose frequency carries a signal affecting some as yet unidentified metabolic activity of the cell (6). Many agents eliciting calcium mobilization are related to control of growth and differentiation.

Human small cell lung cancers (SCLCs) produce bombesin-like peptides (7, 8), which are autocrine growth factors for some human SCLC cell lines (9–11). Bombesin and its human analogue, gastrin-releasing peptide (GRP), are mitogenic for fibroblasts, pulmonary endothelial cells, and a number of other cell lines (12, 13). The growth stimulation produced by these peptides on SCLC cell lines has been shown to be preceded by a series of events including activation of phospholipase C leading to phosphatidylinositol bisphosphate hydrolysis, inositol trisphosphate liberation, diacylglycerol formation, and protein kinase C activation. Those events produce rapid, transient liberation of calcium from internal

stores (14–18) and may lead to cell proliferation with *c-myc* and *c-fos* transcription, but the actual pathways are not delineated (19–21). The GRP-induced rapid increase in intracellular free calcium in some SCLC cell lines can in some cases be blocked by anti-GRP monoclonal antibody, by GRP peptide antagonists, and by substance P derivatives (22–24). The development of indo-1 AM and related fluorescent probes has made it possible to assess calcium flux in individual cells by flow cytometry (25–27).

METHODS AND MATERIALS

Cell Lines. Human SCLC cell lines NCI-H345 and H510 and the large cell lung cancer cell line NCI-H460 were obtained from J. Minna and A. Gazdar (National Cancer Institute). Breast cancer cell lines ZR-75 and MCF-7 were provided by M. Graham and K. Horwitz (University of Colorado Cancer Center). The cells were maintained in serum-free HITES medium (28) for SCLC cell lines or RPMI 1640 medium with 10% fetal calf serum for NCI-H460 and breast cancer cell lines. The Chinese hamster ovary cell line CHO-K1, from our own laboratory, was cultivated in F-12 medium supplemented with 7% fetal calf serum.

The peptides studied, including [Tyr⁴]bombesin, GRP, alytesin, ranatensin, neurotensin, bradykinin, cholecystokinin octapeptide [CCK-(26–33)], gastrin I, arginine vasopressin, litorin, motilin, substance P, and [D-Arg¹, D-Phe⁵, D-Trp^{7,9}, Leu¹¹]substance P, were obtained from Peninsula Laboratories. The terminal amino acid sequences of these peptides are listed in Table 1.

Calcium Assays. Indo-1 AM (Molecular Probes) loading of cells was performed according to the methods of Ransom *et al.* (25) with minor modifications. Mechanically dispersed single-cell suspensions of plateau phase (7–9 days after splitting) cells were suspended at 2.5×10^6 cells per ml in 20 mM Hepes/BSS buffer (20 mM Hepes/140 mM NaCl/5 mM KCl/1 mM MgCl₂/5 mM glucose) adjusted to pH 6.8 at 37°C. The acetoxymethyl ester form of indo-1 (indo-1 AM) at 5 μ M was incubated with the cells for 30 min at 37°C and then diluted 1:1 with 20 mM Hepes/BSS buffer adjusted to pH 7.4 to raise the final pH to ≈ 7.1 followed by a second 30-min incubation at 37°C. The cells were then washed twice in Hepes/BSS buffer, pH 7.4 (37°C) containing dextrose and were resuspended to 1×10^6 cells per ml prior to flow cytometric analysis, which was performed with an EPICS 752 cell sorter (Coulter). UV excitation (80 mW at 360 nm) was delivered by a model INNOVA 90/5 argon ion laser (Coher-

Abbreviations: SCLC, small cell lung cancer; GRP, gastrin-releasing peptide; CCK, cholecystokinin; Bt₂cAMP, N^6, O^2 -dibutyryl adenosine 3',5'-cyclic monophosphate.

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Table 1. C-terminal octapeptide amino acid sequence of the various peptides

Peptide <i>n</i> *	C-terminal octapeptide							
Group A								
Bombesin (6)	Gln Trp	Ala Val	Gly His	Leu Met	-NH ₂			
GRP-(1-27) (19)	His Trp	Ala Val	Gly His	Leu Met	-NH ₂			
Alytesin (6)	Gln Trp	Ala Val	Gly His	Leu Met	-NH ₂			
Ranatensin (3)	Gln Trp	Ala Val	Gly His	Phe Met	-NH ₂			
Litorin (1)	Gln Trp	Ala Val	Gly His	Phe Met	-NH ₂			
Group B								
Gastrin I (9)	Glu Ala	Tyr Gly	Trp Met	Asp Phe	-NH ₂			
CCK-(26-33)	Asp Tyr [†]	Met Gly	Trp Met	Asp Phe	-NH ₂			
Group C								
Neurotensin (5)	Lys Pro	Arg Arg	Pro Tyr	Ile Leu	-C			
Group D								
Bradykinin (1)	Pro Pro	Gly Phe	Ser Pro	Phe Arg	-C			
Group E								
[Arg ⁸]- vasopressin (1)	Tyr Phe	Gln Asn	Cys Pro	Arg Gly	-NH ₂			
Peptides yielding no response with NCI-H345 cells								
Substance P (3)	Pro Gln Gln	Phe Phe	Gly Leu Met	-NH ₂				
Substance P derivative [‡] (3)	Pro D-Phe Gln D-Trp Phe D-Trp Leu Leu	-NH ₂						
Motilin (14)	Glu Lys Glu	Arg Asn	Lys Gly Gln	-C				

*Number of amino acids on the N termini not illustrated—i.e., bombesin has 14 total amino acids.

[†]Tyr (SO₃) form.

[‡][D-Arg¹, D-Phe⁵, D-Trp^{7,9}, Leu¹¹] substance P.

ent, Palo Alto, CA). Violet emission fluorescence intensity (397–417 nm), which is emitted from calcium-bound indo-1, was obtained with a 410-nm band pass filter (Oriol, Stratford, CT) and blue emission fluorescence (480–500 nm), which is emitted from free indo-1, was obtained with a 490 band pass filter (Oriol). Thus, the ratio of the 410-nm fluorescence/490-nm fluorescence constitutes a measure of the changes in calcium ions liberated from intracellular stores in response to various stimuli (26). Viable, loaded cells were distinguished by the machine from debris, dead cells, and unloaded cells by forward angle and 90° light scatter and 490-nm fluorescence. The ratio of 410-nm fluorescence/490-nm fluorescence emission was calculated digitally for each cell by the MDADS hardware and displayed on a linear scale. Data were collected by the MDADS computer including 410-nm and 490-nm fluorescence intensities and the 410-nm fluorescence/490-nm fluorescence intensity ratio was secured as a function of time. For each experiment, measurements were first performed on unstimulated cells followed by the addition of each specific agent and resumption of measurements over a period of time. The data were analyzed by a program developed by P.B.J. Cells were scored as positive if they exhibited a fluorescence ratio at least 10% greater than that of the mean of the unstimulated population.

To determine the reproducibility of the measurements, we performed a variety of control experiments. Repeated flow cytometric analysis of NCI-H345 cells loaded separately with indo-1 AM and stimulated with the same peptide show a mean percentage of responding cells of 54.52 ± 2.84 SD and the mean maximum fluorescence ratio was 1.51 ± 0.045 SD. Biologic variation in the condition of the cells from month to month displayed somewhat greater variability. Thus, cells repeatedly analyzed over a 1-year period demonstrated a mean of the responding cell fraction of 42.3 ± 12.3 SD after administration of 100 nM bombesin.

Ionomycin (Calbiochem), a calcium-specific ionophore, prepared in stock solution at 1 mg/ml with dimethyl sulfoxide, was administered as a single stimulus after each flow cytometric experiment (27). It complexes with calcium ion in

a 1:1 stoichiometry and is capable of extracting calcium ions from an aqueous phase into an organic phase. The response of virtually all of the cells to ionomycin ensured that the adequate indo-1 loading had occurred.

EXPERIMENTAL RESULTS

The Effect of [Tyr⁴]Bombesin on the SCLC Cell Line NCI-H345. The effects of 100 nM [Tyr⁴]bombesin on the intracellular calcium concentration as a function of time is shown in Fig. 1. Fig. 1A shows the 410-nm fluorescence/490-nm fluorescence ratio (free calcium concentration) versus time calculated on the basis of the total cell population. Fig. 1B shows the percentage of responding cells versus time. Within 15 sec of the addition of bombesin there is a transient shift of the 410-nm fluorescence/490-nm fluorescence ratio of intracellular calcium concentration in the responding cells, which returns to baseline values over 1–2 min. There is no evidence of subsequent oscillations in the intracellular calcium concentrations induced by bombesin.

The dose-response relationship of bombesin on calcium flux in Fig. 2 shows that no change in intracellular free calcium ion occurs with bombesin concentrations below 10 nM. At concentrations between 30 and 60 nM, the fraction of responding cells increases with the [Tyr⁴]bombesin concentration, while the time necessary to achieve the maximum value decreases. Between 60 and 200 nM [Tyr⁴]bombesin, the effect achieved is maximal. At these concentrations the maximum percent of responding cells is between 40% and 50% and the maximum number of cells responding is achieved ≈ 25 sec after the stimulus is applied. Rates of decay of free calcium ion concentration after achievement of the maximum value were rapid and relatively independent of the concentrations of the eliciting agent.

The Effect of Other Agents on Calcium Ion Release from NCI-H345 SCLC Cells. The effects on NCI-H345 elicited by the other peptides listed in Table 1 applied at a concentration of 100 nM are shown in Table 2. The highly related peptides

FIG. 1. (A) Time course of the Ca²⁺ flux response to [Tyr⁴]bombesin addition (arrow). The mean fluorescence ratio of the total cell population is shown. (B) Time course of the percentage of responding cells exhibiting a fluorescence ratio greater than the critical threshold.

FIG. 2. Response of NCI-H345 cells to [Tyr4]bombesin in various concentrations. The percentage of cells responding over 1–100 sec is shown immediately after application of the indicated stimulus. Each point represents an analysis of 800–1200 cells.

and partial peptides of group A, including GRP-(1–27), GRP-(14–27), GRP-(20–27), alytesin, ranatensin, and litorin, elicited calcium ion mobilization patterns similar in magnitude and duration to that of bombesin. The partial peptide GRP-(1–16) elicited no response. In the responses generated by the series of complete and partial GRP peptides, the positive result with GRP-(20–27) and the failure of GRP-(1–16) to elicit a response show the C-terminal octapeptide is necessary and sufficient for biologic activity. The group B and C peptides, which have no peptide sequence homology to the group A peptides, elicited calcium flux changes of similar degree and magnitude to the group A bombesin-related peptides. Peptides from groups D (bradykinin) and E (arginine-8 vasopressin), which have no sequence homology to peptides from groups A–C, produced positive responses but of lower magnitude than peptides from groups A–C. Motilin, substance P,

Table 2. $[Ca^{2+}]_i$ response of NCI-H345 cells to various neuropeptide stimuli

Peptide	Concentration	Maximum % cells responding	Ratio
Group A			
Bombesin	100	42	1.43
GRP-(1–27)	100	45	1.35
GRP-(14–27)	100	45	1.35
GRP-(20–27)	100	45	1.35
GRP-(1–16)	100	0	1.00
Alytesin	100	45	1.35
Ranatensin	100	45	1.35
Litorin	100	45	1.35
Group B			
Gastrin I	100	60	1.43
CCK-(26–33)	100	60	1.41
Group C			
Neurotensin	100	60	1.38
Group D			
Bradykinin	100	30	1.25
Group E			
[Arg ⁸]vasopressin	100	19	1.12
Other agents			
Substance P	100	0	1.0
Substance P derivative	100	0	1.0
Motilin	100	0	1.0
Fetal calf serum	1%	70	1.93
Ionomycin	4 μ M	100	4.0

$[Ca^{2+}]_i$, internal calcium concentration. The ratio is the mean fluorescence of 410-nm UV light divided by 490-nm fluorescence of the total cell population at the point of maximal cellular response. Resting cell ratio = 1.0; maximal stimulation = 4.0. Concentration is nM unless indicated otherwise.

and substance P derivative elicited no calcium response from NCI-H345 cells.

A number of other growth factors and hormones that may potentially serve as mitogens for SCLC or participate in second messenger signaling were tested with the NCI-H345 cells. No calcium response was obtained with Bt₂cAMP, insulin, transferrin, selenium, estradiol, hydrocortisone, physlaemin, somatostatin, nerve growth factor, transforming growth factor α , transforming growth factor β , epidermal growth factor, platelet-derived growth factor, interleukin 1, interleukin 3, granulocyte–macrophage colony-stimulating factors, and macrophage-stimulating factors.

The effects of rabbit and fetal calf serum in eliciting calcium flux responses from the SCLC cells were also evaluated (Table 2). Both sera in all concentrations varying between 0.1% and 1% elicited calcium flux in as much as 70% of the cell population.

The Effects of Multiple Peptides Administered Simultaneously and Sequentially on Calcium Ion Release from NCI-H345 Cell Lines. *Simultaneous addition of multiple peptides.* The simultaneous addition of any combination of active peptides of Table 1, group A (bombesin, GRP, alytesin, ranatensin, or litorin), each in a concentration eliciting maximal response, produced the same response as any single peptide alone (Table 3). Similarly, the simultaneous combination of both of the peptides of group B [gastrin I and CCK-(26–33)] produced a response of equal magnitude to that of each single peptide (Table 3). In contrast, combinations of any two peptides from different active groups of Table 1 administered simultaneously, elicited a calcium flux of greater magnitude than either single peptide alone (Table 3 and Fig. 3A). Thus, structurally unrelated peptides from groups A–E appear to activate different calcium reservoirs while structurally homologous

Table 3. $[Ca^{2+}]_i$ representative responses to single neuropeptide stimuli or serum alone compared to simultaneous or sequential application of multiple stimuli for NCI-H345 cells

	Maximum % cells responding	Final mean fluorescence ratio
Single peptide		
BN	36	1.30
RN	42	1.33
CCK-(26–33)	46	1.40
G	45	1.39
NT	32	1.28
1% FCS	62	1.66
Simultaneous peptide stimulation		
BN + RN	42	1.31
BN + CCK-(26–33)	63	1.61
BN + NT	51	1.51
BN + 1% FCS	70	1.77
BN + NT + CCK-(26–33)	63	1.68
BN + NT + CCK-(26–33) + 1% FCS	78	1.98
Sequential peptide stimulations		
BN $\rightarrow$ BN	36 $\rightarrow$ 0	1.0
BN $\rightarrow$ RN	36 $\rightarrow$ 0	1.0
BN $\rightarrow$ CCK-(26–33)	36 $\rightarrow$ 48	1.37
BN $\rightarrow$ NT	36 $\rightarrow$ 35	1.26
BN $\rightarrow$ 1% FCS	36 $\rightarrow$ 69	1.60
CCK-(26–33) $\rightarrow$ CCK-(26–33)	46 $\rightarrow$ 0	1.0
CCK-(26–33) $\rightarrow$ G	46 $\rightarrow$ 0	1.0
CCK-(26–33) $\rightarrow$ BN	46 $\rightarrow$ 36	1.25
NT $\rightarrow$ NT	32 $\rightarrow$ 0	1.0
NT $\rightarrow$ BN	32 $\rightarrow$ 36	1.29
NT $\rightarrow$ CCK-(26–33)	32 $\rightarrow$ 41	1.36

$[Ca^{2+}]_i$, internal calcium concentration; BN, bombesin; RN, ranatensin; G, gastrin I; NT, neurotensin; FCS, fetal calf serum. All peptide additions were at 100 nM final concentration.

FIG. 3. Typical flow cytometric analyses of calcium response to single, simultaneous, and sequential peptide additions to NCI-H345 cells. Each peptide alone or in combination was administered at 100 nM. Fetal calf serum (FCS) was applied at 1%. (A) Plots show the combined effect of two or more peptides from different groups and FCS applied simultaneously. The elicited maximum values are always greater than those from the single peptides, as shown in B and C. Simultaneous combinations of peptides from the same group were always identical to those of each peptide alone within experimental uncertainty. (B) Plots illustrate the elicited calcium response to [Tyr⁴]bombesin (BN), neurotensin (NT), CCK-(26-33), and 1% FCS followed by refractoriness to a second identical stimulus applied 100 sec later. (C) Plots show the calcium response to sequential administration of two structurally dissimilar peptide agonists (i.e., from different groups) applied 100 sec apart. Each point of peptide application is indicated by arrows.

ones do not. Similarly, combinations of three or more structurally unrelated peptides increased the magnitude of the response still further, although not in a quantitatively additive manner. Thus, 78% of the cells responded to the combination of bombesin, CCK-(26-33), neurotensin, and 1% fetal calf serum (Table 3 and Fig. 3A). Both the percentage of cells responding and the magnitude of the response were not altered for simultaneous addition of peptides of the same class, but they did increase when peptides from different classes were used. These results imply that a set of alternative and at least partly mutually exclusive pathways exist for the mobilization of intracellular calcium stores.

Sequential additions of peptides. The specificity of separate pathways is reinforced by experiments in which optimal concentrations of peptides were administered sequentially, the second addition occurring after the calcium ion concentration had returned to normal. Sequential administration of the same peptide (e.g., bombesin followed by bombesin) produced no new response to the second addition (Table 3 and Fig. 3B). In a series of experiments in which a second bombesin stimulus was added at intervals varying from 2 min to 72 hr after the first stimulus, it was found that cells were refractory to a second bombesin addition for 1-6 hr and recovered their full calcium stimulatory pattern by 24 hr. Moreover, an initial suboptimal stimulus eliciting no calcium change at 10 nM concentrations of [Tyr⁴]bombesin or a structurally homologous peptide was also capable of completely inhibiting response to a maximal stimulus (100 nM) administered 3 min later. Sequential administration of peptides of structurally different groups exhibited no mutually inhibitory action, so that the cells responded to each new stimulus in a manner identical to that elicited when administered alone (Table 3 and Fig. 3C).

These data indicate that cells responding to these peptides are refractory to subsequent stimuli by peptides sharing carboxyl-terminal homology, even after the intracellular calcium concentration (410-nm fluorescence/490-nm fluorescence) has returned to its original condition, suggesting the possibility of inhibition at the receptor or at later points in the pathway. Thus, different classes of calcium flux stimulants operate through different pathways whose effects are at least partially additive. The experiments described here furnish means for determining whether any two calcium flux stimulants belong to the same or different classes.

Responses of other cancer cell lines. The effect of the various agents was studied on additional human cell lines derived from lung cancer, breast cancer, and on CHO-K1, a malignant Chinese hamster ovary cell line that has been used

as a model system for a number of studies (29). Considerable differences in the pattern of response to these peptides were exhibited by different cell lines as indicated in Table 4. Thus, two breast adenocarcinoma cell lines did not respond to any of these peptides or to fetal calf serum (Table 4). CHO-K1 cells responded to all five classes of neuropeptides to a degree similar to the SCLC cell lines. Calcium mobilization occurred in typical fashion to Bt₂cAMP, an agent that specifically produces reverse transformation in this cell (29). cAMP did not, however, cause calcium mobilization in other cell lines. These data demonstrate heterogeneity of calcium flux response to various agonists in different cancer cell lines, a heterogeneity that may be due to differences in receptor composition, metabolic pathways beyond the receptor stage, or both.

DISCUSSION

The data presented here demonstrate that intracellular calcium mobilization in response to a variety of neuropeptides is a common property of several cancer cell lines but displays cell-specific patterns. The responsiveness of two SCLC cell lines was greater than the responsiveness of a non-SCLC cell line; breast cancer cell lines were unresponsive. These data parallel the neuroendocrine features of SCLC that overlap in some non-SCLCs and are absent in breast cancers. The responsiveness of CHO-K1 cells shows that other types of cells also can share this general signal pathway.

The flow cytometric method for evaluating individual cells loaded with the calcium indicator indo-1 AM allowed for

Table 4. Calcium response of cell lines of different origin to different stimuli

Cell lines	% cells responding to						
	A	B	C	D	E	Bt ₂ cAMP	1% FCS
SCLC							
NCI-H345	42	47	45	30	19	0	70
NCI-H510	25	67	10	14	47	0	73
Large cell lung cancer							
NCI-H460	0	23	0	28	0	0	29
Breast cancer							
ZR-75	0	0	0	0	0	0	0
MCF-7	0	0	0	0	0	0	0
Ovary							
CHO-K1	48	29	42	28	17	29	71

A-E represent peptide groups A-E. FCS, fetal calf serum. Bt₂cAMP concentration was 1 mM.

quantification of response with respect to both the percentage of cells responding and the magnitude of the calcium response. In most instances, only a fraction of cells responded to each stimulus and appropriate combinations of stimuli often produced increases in the fraction of responding cells that were not quantitatively additive. These results may reflect the fact that calcium responsiveness varies with the location of cells within the cell cycle, with the degree of receptor expression that is regulated by the receptor-ligand interaction and other factors, and by other steps beyond the receptor in the pathway leading to calcium ion flux.

The neuropeptides evaluated can be grouped into five classes based on their amino acid structure and their patterns of calcium response to simultaneous and sequential stimuli. When peptides of a single class that share close homology in the C-terminal octapeptides were added simultaneously, and in concentrations designed to produce maximal response, the combined response was quantitatively identical to that of a single peptide. Furthermore, there is a refractory period after response to stimulus, that lasts beyond the return of calcium concentrations to normal values. The refractory period lasts at least 6 hr and cells are refractory to all peptides of the same class during this period. However, consecutive application of a peptide of a different class elicits a calcium response typical for that peptide applied *de novo* to unstimulated cells. When peptides of different classes are applied simultaneously, the calcium response is greater than that observed with either peptide alone, although again the increase is not quantitatively additive. These results demonstrate that when a peptide-induced calcium flux terminates, the cell is not in the same state it occupied before the stimulus. Other studies have demonstrated that the binding of bombesin to its receptor on 3T3 cells did not lead to receptor down-regulation (23). Our data indicate an inhibitory action but it is uncertain whether this results from receptor down-regulation or inhibition of a subsequent portion of the pathway. The results observed imply that the stimulation of calcium flux constitutes a family of different cellular activations, each controlled by a separate pathway. Agonists of related structure trigger the same pathway while those of different structure stimulate alternative pathways, which also lead to the release of calcium. It may be that these pathways are distinct only in their respective receptors or that there are also differences in subsequent steps including the coupling to other proteins and calcium reservoirs. Thus, guanine nucleotide binding proteins, phospholipase C, and inositol trisphosphate participate in this pathway and may be coupled in the same or different ways to other receptors and subsequent metabolic targets. Clearly, there are alternative growth and differentiation pathways that use the triggering of calcium flux phenomena when stimulated by the appropriate agonists. Similarly, different metabolic states each associated with a calcium flux can be triggered in cells in response to particular growth and differentiation agonists.

The response induced by Bt₂cAMP in CHO-K1 cells is noteworthy since this is a model cell studied in the reverse transformation reaction and its genetic regulatory functions (29, 30). CHO-K1 cells alone, of all the cells studied here, demonstrated a calcium flux response to cAMP derivatives, agents that also cause it to lose its malignant characteristics and regain its normal differentiated phenotype.

The data presented here show that multiple neuropeptides stimulate release of calcium from lung cancer, but not breast cancer, cell lines. Further elucidation of the specific pathways involved may lead to a better understanding of growth

and differentiation pathways and new approaches to cancer treatment.

Thanks are due to Lisa Furmanski for technical assistance. This work was supported in part by Grants U01 CA 46088-01 and 1 P30 CA 46934-02 from the National Cancer Institute, National Institutes of Health; the Cancer League of Colorado; The Lucille P. Markey Charitable Trust; and The Raphael Levy Memorial Foundation. T.T.P. is an American Cancer Society Professor.

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