VASOACTIVE INTESTINAL PEPTIDE (VIP) CONTROL OF GLYCOGENOLYSIS IN THE HUMAN COLON CARCINOMA CELL LINE HT-29 IN CULTURE

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1. Introduction

Human intestine malignant epithelial cells store glycogen in vivo [1,2] and in vitro [2,3]. Studies performed in vitro with established cell lines have shown that the quantity of glycogen stored varies from one cell line to another, is closely related to the process of cell growth, and represents a characteristic feature of each cell line [2,3]. These same cell lines have been shown to possess an adenylate cyclase system which is highly sensitive to the vasoactive intestinal peptide (VIP) [4,5]. In view of the known effect of cyclic AMP on glycogenolysis in various systems [6], it seemed justified to investigate whether VIP was able to stimulate glycogenolysis in such cell lines in culture. The cell line HT-29 was selected for this study. It originates from a well-differentiated colon adenocarcinoma [7], and was chosen for the following reasons:

(1) Its cyclic AMP production system is highly sensitive to VIP [4,5];
(2) VIP elicits an activation of cyclic AMP-dependent protein kinases in these cells [8];
(3) Their glycogen content falls in the middle of the range of the values found in the different intestine cell lines so far investigated [2,3].

Our data show that VIP provokes a cascade of events involving an increase of cyclic AMP, an activation of glycogen phosphorylase, and a subsequent glycogenolysis in monolayers of exponentially growing HT-29 cells.

2. Materials and methods

2.1. *Cell cultures and incubation procedures*

The cells were seeded at 0.5×10^6 cells/flask in 25 cm^2 plastic flasks (Corning) and cultured at 37°C in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY), in an atmosphere of 90% air/10% CO_2. The medium was changed after 60 h and VIP added to the culture medium 12 h later. The incubation was performed at 37°C. The number of cells at the moment of the assay was $\sim 4 \times 10^6$ cells/flask (= 1 mg protein). For cyclic AMP and glycogen phosphorylase assays, the culture medium was rapidly removed, the cells rapidly washed with ice-cold phosphate-buffered saline Ca^{2+}- and Mg^{2+}-free (pH 7.2) and immediately snap frozen by flotation of the flasks on a bath of liquid nitrogen. The flasks were stored at −70°C for subsequent extraction. For the glycogen and protein assays, the cells were harvested with trypsin 0.25%, divided equally, centrifuged (5 min, 4°C, $100 \times g$) and washed twice in ice-cold phosphate-buffered saline, and stored at −70°C.

2.2. *Glycogen and protein quantitative assays*

Glycogen was extracted from the cells and measured with anthrone (Sigma) as in [2–4], according to the method in [9], as modified [10]. The method with anthrone was found to have the same sensitivity as that using glucose oxydase. The protein content was measured by the method in [11].

2.3. *Glycogen phosphorylase assay*

For enzyme extraction, an ice-cold buffer (pH 7.4) containing 62.5 mM glycyl-glycine, 6.25 mM EDTA, 125 mM NaF, 0.5 M saccharose, and 5 mM dithiothreitol was added to the flasks, and the frozen cell layers were scraped with a rubber policeman. The volume of the buffer was adjusted to ~3 mg cell protein/ml. The cells were disrupted by multiple passages through a 26 G needle, and centrifuged (30 min, 4°C, 800 × *g*). Glycogen phosphorylase was assayed according to [12], modified by the addition of caffeine (1 mM) [13], by measuring the incorporation of [^{14}C]glucose-1-phosphate into rabbit liver glycogen (Sigma), in the presence of AMP (Sigma) for total enzyme activity, and without AMP for phosphorylase *a* activity.

2.4. *Cyclic AMP assay*

Cyclic AMP was extracted from the cells by thawing and scraping the frozen cell layers in methanol at room temperature. The methanol extract was centrifuged (15 min, 4°C, 800 × *g*) and evaporated. Cyclic AMP was measured by the modified [14] radioimmunological technique of [15].

3. Results and discussion

In [4], with detached cells, at 15°C, and in the presence of a phosphodiesterase inhibitor, the optimum concentration of VIP necessary to elicit a maximum accumulation of cyclic AMP in HT-29 cells was 10^{-9} M. As this study involved the use of monolayers of exponentially growing cells, at 37°C, and no phosphodiesterase inhibitor, a dose–effect study of VIP on glycogenolysis was done before further investigation. Fig.1 shows that under such experimental conditions the optimum concentration of VIP required to elicit a maximum glycogenolytic effect in HT-29 cells was 10^{-8} M.

In all 3 expt performed at different cell passages, VIP at 10^{-8} M induced the same changes in the same invariable order of sequence (fig.2): cyclic AMP rapidly increased, from a basal value of 0.77 ± 0.05 pmol/mg protein up to a peak (~40-times higher than the basal) at 30 s. This peak was followed by a rapid decrease; a plateau was reached after 5 min which remained constant for 3 h and was still 10-times higher than the basal value. The activity of glycogen phosphorylase *a* increased from the initial value of

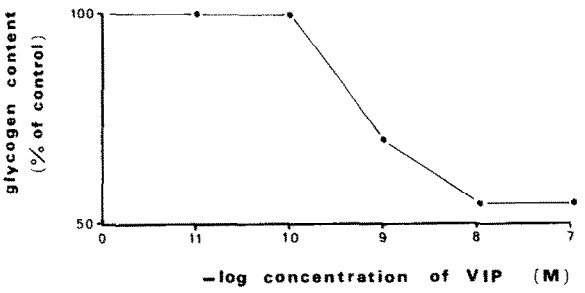

Fig.1. Dose–effect of VIP on glycogenolysis in monolayers of exponentially growing HT-29 cells. Glycogen was measured after 60 min incubation. Each point is the mean of 3 separate expt. SD values (not shown) are <10%.

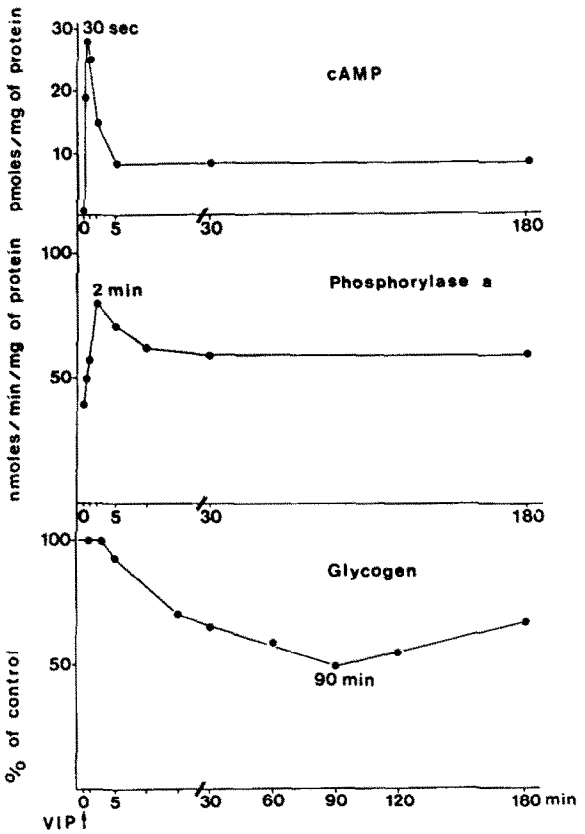

Fig.2. Effect of VIP (10^{-8} M) on cyclic AMP accumulation (top), glycogen phosphorylase *a* activity (middle), and intracellular glycogen concentration (bottom) in monolayers of exponentially growing HT-29 cells. Each point is the mean of 3 separate expt.

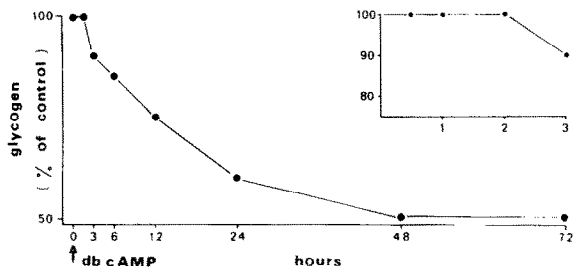

Fig.3. Glycogenolytic effect of dibutyryl cyclic AMP (10^{-3} M) in monolayers of exponentially growing HT-29 cells. Each point is the mean of 4 different expt. SD values (not shown) are <10%.

40 ± 3 nmol . min^{-1} . mg $protein^{-1}$ to twice this value 2 min later. The activity gradually fell off; after 30 min a plateau was reached which again remained stable for 3 h and stayed higher than the control. These variations of the *a* form of glycogen phosphorylase contrasted with the constancy of the total activity of the enzyme (100.9 ± 5.2 nmol . min^{-1} . mg $protein^{-1}$, not shown in fig.2). The concentration of glycogen began to decrease after 5 min. This decrease was slow, starting from 23.4 ± 0.4 μg glycogen/mg protein, and dropped to a minimum value of 11.7 ± 0.9 μg at 90 min. At 6 h after the addition of VIP (not shown in fig.2), the concentration of cyclic AMP had decreased to double the basal value, the activity of phosphorylase *a* was back to the control value, while the concentration of glycogen still remained lower than the control (80%). The transient effect of VIP was not due to its degradation in the culture medium. Indeed, the concentration of VIP in the culture medium, after 6 h, was still sufficient to elicit a maximum stimulation of cyclic AMP accumulation in fresh HT-29 cells. Therefore, further studies need to be undertaken to check if this transient effect is due to a desensitisation process.

The addition of dibutyryl cyclic AMP (10^{-3} M) to the culture medium produced the same degree of glycogenolysis as that induced by VIP (fig.3). The glycogenolytic effect produced by dibutyryl cyclic AMP was however much slower, starting after 2 h, and reaching its maximum value at 24–48 h. This delayed effect is probably due to a low permeation of dibutyryl cyclic AMP into the cells, in the same way as has been described in HeLa cells [17].

These data confirm that HT-29 cells retain an adenylate cyclase system with a sensitivity to VIP similar to that of normal human colocytes [4,5,16]. They demonstrate that these cells also possess a functional cyclic AMP-dependent metabolic pathway of glycogenolysis.

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References

[1] Rousset, M., Robine-Léon, S., Dussaulx, E., Chevalier, G. and Zweibaum, A. (1979) Front. Gastrointest. Res. 4, 80–85.
[2] Rousset, M., Dussaulx, E., Chevalier, G. and Zweibaum, A. (1980) J. Natl. Cancer Inst. 65, 885–889.
[3] Rousset, M., Chevalier, G., Rousset, J. P., Dussaulx, E. and Zweibaum, A. (1979) Cancer Res. 39, 531–534.
[4] Laburthe, M., Rousset, M., Boissard, C., Chevalier, G., Zweibaum, A. and Rosselin, G. (1978) Proc. Natl. Acad. Sci. USA 75, 2772–2775.
[5] Laburthe, M., Rousset, M., Chevalier, G., Boissard, C., Dupont, C., Zweibaum, A. and Rosselin, G. (1980) Cancer Res. 40, 2529–2533.
[6] Soderling, T. R. and Parks, C. R. (1974) Adv. Cyclic Nucl. Res. 4, 283–333.
[7] Fogh, J. and Trempe, G. (1975) in: Human Tumor Cells *In Vitro* (Fogh, J. ed) pp. 115–141, Plenum, New York.
[8] Marvaldi, J., Mangeat, P., Ait Ahmed, O., Coeroli, C. and Marchis-Mouren, G. (1979) Biochim. Biophys. Acta 588, 12–19.
[9] Seifter, S., Dayton, S., Novic, B. and Muntweiler, E. (1950) Arch. Biochem. Biophys. 25, 191–200.
[10] Van Handel, E. (1965) Anal. Biochem. 11, 256–265.
[11] Lowry, O., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[12] Wang, P. and Esman, V. (1972) Anal. Biochem. 47, 495–500.
[13] Van de Werve, G., Van den Berghe, G. and Hers, H. G. (1974) Eur. J. Biochem. 41, 97–102.
[14] Rosselin, G., Freychet, P., Fouchereau, M., Rancon, F. and Broer, Y. (1974) Horm. Metab. Res. 5, 72–78.
[15] Steiner, A. L., Pagliera, A. S., Chase, L. R. and Kipnis, D. M. (1972) J. Biol. Chem. 247, 1106–1113.
[16] Dupont, C., Laburthe, M., Broyart, J. P., Bataille, D. and Rosselin, G. (1980) Eur. J. Clin. Invest. 10, 67–76.
[17] Kaukel, E. and Hilz, H. (1972) Biochem. Biophys. Res. Commun. 46, 1011–1018.