# TRANSFER OF THE HORMONE RECEPTOR FOR VASO-INTESTINAL PEPTIDE TO AN ADENYLATE CYCLASE SYSTEM IN ANOTHER CELL

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## 1. Introduction

The β-adrenergic receptor on the turkey erythrocyte was shown to couple with the adenylate cyclase system of the Friend erythroleukemia cell when the membranes of the two cells are fused with each other by Sendai virus [1]. Prior to cell fusion the adenylate cyclase system in the turkey erythrocyte was irreversibly inactivated by *N*-ethylmaleimide (NEM) or by heat so that this cell could contribute only the hormone receptor but not the enzyme. The work was subsequently extended to hybridize the β-adrenergic receptor and also the prostaglandin $E_1$ receptor with adenylate cyclase systems of various types of cultured cells [2]. These experiments suggested that the receptors tested exist in the cell membrane as independent units which freely dissociate from the adenylate cyclase system and that receptors and enzyme, originating from various tissues and from different animal species, are compatible with each other in forming a functional complex. However, these conclusions were based only on testing the receptors for two hormones of low molecular weight.

Receptors for the much larger and structurally different molecules, the peptide hormones, might have rather different properties. It had, for instance, been demonstrated that receptors for peptide hormones are quite susceptible to trypsin while the β-adrenergic receptor in the same cells was not [3]. Because of these considerations and because of the great physiological importance of the peptide hormones, it seemed essential to learn whether their receptors too are freely transferable to the adenylate cyclase system of other cells. For this purpose a cell line originating from a human colon carcinoma (HT29) [4], possessing a receptor [5] for the vaso-active intestinal peptide hormone (VIP) was chosen. This cell line has been shown in studies of hormone binding to possess a considerable number of VIP receptors and to respond to VIP with a dramatic increase in the level of intracellular cyclic AMP [5,6]. Thus the HT29 cell seemed to fulfill the prerequisites for an attempt to transfer the receptor for a peptide hormone.

It should be noted that VIP is a well-defined hormone of known sequence [7], which has been prepared also synthetically [8]. It is found at high concentration in the gut but recently was also located in brain cortex and hypothalamus [9]. VIP receptors were characterized in pancreas [5,10,11], gut [12], liver [13,14], fat cells [14] and in the HT29 cell line [6].

## 2. Materials and methods

For the current experiments cultures of HT29 were grown in Roux glass bottles for 6–8 days at 37°C in Dulbecco's modified Eagle medium containing 10% fetal calf serum. The cells were suspended by treating 3 min with crystalline proteases as in [2]. To terminate proteolysis [2] *p*-methylsulfonyl fluoride, $10^{-5}$ M was added together with soybean trypsin inhibitor, 1 mg/ml. The protease inhibitors were added at all subsequent steps of preparation of the HT29 cell suspension. The cells were washed and

suspended in $Na^+$ medium [2]. Aliquots were treated with 5 mM NEM for 10 min at 4°C to inactivate the adenylate cyclase [1]. Five volumes of 14 mM mercaptoethanol were subsequently added at 0°C. The suspension was kept 3 min at 0°C followed by 10 min at 25°C. After centrifugation the cells were suspended in $Na^+$ medium.

Growth of T3C12 Friend erythroleukemia (Fc) cells [1] and their fusion with HT29 cells was performed as in [2]. Activation of adenylate cyclase by VIP was measured after cell fusion on cell ghosts prepared by hypotonic lysis [1].

## 3. Results and discussion

Fifteen experiments of fusion of NEM-treated HT29 cells with Fc cells were performed. In all experiments there was a definite enhancement of adenylate cyclase activity due to the addition of VIP to the assay system. The increase in rate ranged from 2–5-fold over the basal activity. A representative experiment is shown in fig.1.

Controls of Fc cells fused with each other or of NEM-treated HT29 cells did not show any stimulation of adenylate cyclase by VIP. Fc cells were also checked for specific VIP binding and none was detected. It can therefore be concluded that the activation of adenylate cyclase by VIP after fusion of NEM-treated HT29 cells with Fc cells is due to the transfer of the VIP receptor to the adenylate cyclase system of the other cell. The affinity of the receptor for VIP seems not to change when it is transferred to the heterologous adenylate cyclase of the Fc cells (table 1). The same observation holds with regard to

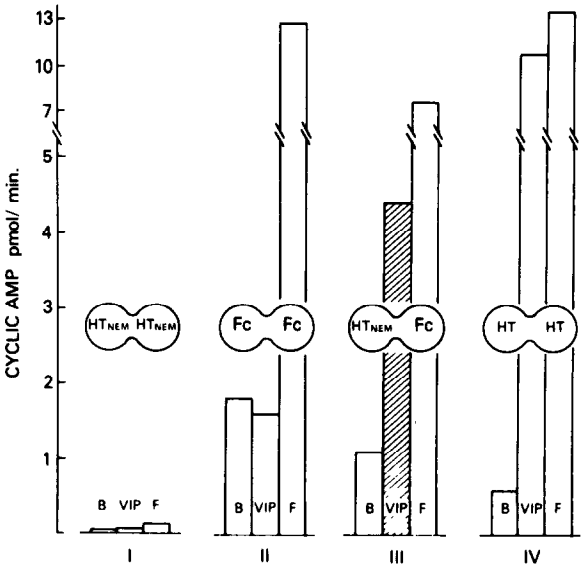


Fig.1. Transfer of the VIP receptor of HT29 cells to the adenylate cyclase of Fc cells by cell fusion. Adenylate cyclase activities are shown for cell ghosts prepared by hypotonic lysis after cell fusion [1,2]. Fusion systems were: (I) NEM-treated HT29 cells, $1 \times 10^7$ fused with each other by Sendai virus, 700 haemagglutinating units (HAU); (II) Fc cells, $3 \times 10^6$ fused with each other, virus 200 HAU; (III) heterologous fusion of HT29 NEM-treated cells, $1.2 \times 10^7$ with Fc cells, $3 \times 10^6$ by virus, 1000 HAU; (IV) untreated, HT29 cells, $2.2 \times 10^6$ fused with each other by virus, 200 HAU. The cell types which participate in fusion are symbolized in the figure by fused circles in each set of columns. At the base of each column the activating agent is specified: B, basal activity; porcine VIP, 20 nM in 0.1% bovine serum albumin; F, fluoride, 10 mM. The response of the hormone receptor transferred to a heterologous system is emphasized by a hatched column. Adenylate cyclase activities shown were measured [15] in duplicate on cell ghosts equivalent to 1/7th of the cells in the original fusion system above. Incubation was 10 min 37°C.

Table 1
Hormone concentration producing half maximal activation of adenylate cyclase in fused cell membranes

| Cells in fusion | VIP (nM) | Secretin (nM) |
|---|---|---|
| HT29–HT29 | 3 | 2000 |
| $HT29_{NEM}$–Fc | 2 | 1000 |

Fusion systems were prepared as in fig.1. Adenylate cyclase activities were determined on cell ghosts in the presence of various hormone concentrations. The half maximal concentration was calculated from a Lineweaver-Burk plot

the closely-related hormone, secretin, which apparently acts on the VIP receptor [5,6,10,11] when applied at a very high concentration. Thus the specificity of the receptor also appears to remain unaltered during transfer.

The experiments clearly show that the receptors for a peptide hormone couple to the adenylate cyclase of another cell as demonstrated for the β-adrenergic and $PGE_1$ receptors [1,2]. It is therefore suggested that the receptors are composed of a

hormone-recognising part which is highly specific and a constant fragment, common to all these receptors, which couples to the adenylate cyclase system. It seems quite likely that the different hormone receptors evolved during evolution from a common ancestor receptor molecule by mutations in the recognition site.

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