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Growth stimulatory effect of pancreatic spasmolytic polypeptide on cultured colon and breast tumor cells

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The effects of a novel polypeptide, pancreatic spasmolytic polypeptide (PSP) on a colon carcinoma cell line (HCT 116) were examined. PSP stimulated the incorporation of [^{3}H]thymidine into HCT 116 cells as well as cell proliferation in a dose-dependent manner. Maximal increase in [^{3}H]thymidine incorporation of 50–60% occurred at 3–300 μM PSP. The VIP-mediated-increase in cAMP levels was reduced by PSP at >1 μM concentrations. PSP is highly homologous to the estrogen-induced pS2 protein in MCF-7 breast cancer cells. We find that PSP also enhanced [^{3}H]thymidine incorporation in MCF-7 cells. These findings indicate for the first time that PSP has growth stimulatory properties.

Protein, pS2; cyclic AMP level; Vasoactive intestinal polypeptide

1. INTRODUCTION

Pancreatic spasmolytic polypeptide (PSP, 11 700 Da) was first isolated from porcine pancreatic extracts as a novel peptide present in large amounts in a side-fraction from the production of porcine insulin [1]. Purified PSP has been shown to inhibit intestinal motility and block gastrin-induced gastric acid secretion [2]. The high concentration of PSP in pancreatic juice after stimulation with secretin and/or pancreozymin [3], its extraordinary stability against proteolytic degradation [1], and the presence of PSP receptors on intestinal epithelial cells [4,5] suggest a unique, intraluminal physiological mode of action for PSP.

PSP displays a high degree of homology to the pS2 protein, an abundant estrogen-induced protein of unknown function, secreted by MCF-7 human breast cancer cell line [6–9]. Similar to PSP [10], pS2 [11] has a high number of cysteine residues whose arrangement bears resemblance to those in insulin-like growth factors suggesting a possible growth factor activity for these peptides [7,11,12]. Also, a novel secretory protein expressed in frog skin, which is known to be a rich source of a variety of physiologically active peptides, is highly homologous to PSP [13]. In this study we have examined the effect of PSP on the growth of cultured human colon carcinoma cells (HCT 116) and on MCF-7 breast tumor cells.

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Abbreviations: PSP, pancreatic spasmolytic polypeptide; VIP, vasoactive intestinal polypeptide; FBS, fetal bovine serum; EGF, epidermal growth factor; TCA, trichloroacetic acid

2. MATERIALS AND METHODS

Porcine PSP was purified as previously described [1]. HCT 116 human colon carcinoma cells were established in culture as described previously [14]. HCT 116 cells have been adapted to growth in serum-free medium which was McCoy's 5A containing twice the normal concentration of sodium pyruvate, vitamins and amino acids. Also added are transferrin (4 μg/ml), insulin 20 μg/ml), EGF (10 ng/ml), sodium selenite (0.4 nM), hydrocortisone (2 μg/ml) and triiodothyronine (0.4 nM). MCF-7 human breast cancer cells were obtained from Dr George Todaro (Seattle, USA) and maintained in McCoy's 5A medium supplemented with 10% fetal bovine serum (FBS).

In the [^{3}H]thymidine incorporation assay, 4 h prior to

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harvest, [^{3}H]thymidine (Amersham, spec. act. 60 Ci/mmol, 0.5 μCi or 1 μCi/well as indicated) was added to each well in a 24-well plate. At the time of harvest, conditioned medium was aspirated and 500 μl of 10% TCA was added. After 10 min, the TCA solution was removed, cells solubilized in 500 μl of 2 N NaOH which was then transferred to 10 ml scintillation cocktail and counted in a Beckman scintillation counter.

To determine cAMP levels HCT 116 cells were grown in 75 cm^2 flasks in serum-free, growth-factor supplemented medium (see above). At late log phase of growth cells were harvested using Ca^{2+}, Mg^{2+}-free Hanks balanced salt solution containing 6 mM EDTA. Cells were spun down, washed once in incubation buffer (1 mg/ml BSA, 1 mM dithiothreitol, 1 mM theophylline, Tris-HCl, pH 7.5) and then enumerated using a hemocytometer. Viability of cells as determined by trypan blue exclusion was >98%. Reaction mixture of total volume 100 μl contained 40 μl of cell suspension (0.6 × 10^6 cells), 30 μl PSP and/or 30 μl VIP (as indicated) in incubation buffer. After 15 min, the reaction was stopped and cAMP determined using the Amersham cAMP assay kit as described previously [15].

Statistical analysis was done by paired Students' *t*-test. Designations are * for $P<0.05$ and ** for $P<0.025$.

3. RESULTS

3.1. *Stimulation of [^{3}H]thymidine incorporation by PSP*

PSP enhanced [^{3}H]thymidine incorporation into HCT 116 colon carcinoma cells in a dose-dependent manner (fig. 1). Stimulation could be observed within 48 h of treatment at low nanomolar concentrations of PSP with a maximal increase of between 50 and 60% over control occurring in the 3–300 μM range. PSP also increased the cell number in a dose-dependent manner with a maximal increase of 60% over untreated control cultures at a concentration of 0.3 μM PSP (not shown).

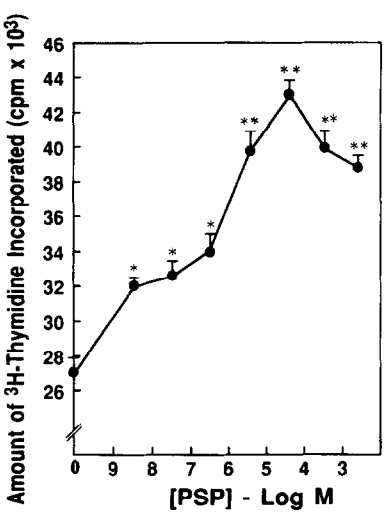

Fig.1. Increased [^{3}H]thymidine-incorporation induced by PSP in cultured colon carcinoma cells. HCT 116 cells were plated in triplicate at 1 × 10^4 cells/well in 24-well plates. 2 days later when cells were 50–60% confluent, conditioned medium was removed and PSP was added to McCoy's 5A medium (without any other growth factor) at the indicated concentrations. Cells were harvested 48 h later. 4 h prior to harvest 1 μCi [^{3}H]-thymidine was added per well. Values shown are mean ± SD of 4 experiments.

Breat tumor cells MCF-7 also responded to PSP with increased [^{3}H]thymidine incorporation (fig. 2). About 20% stimulation was observed at 3 nM PSP and a maximal increase of 50–60% at 0.03–3 μM PSP.

3.2. *Effect of PSP on cAMP levels in colon tumor cells*

PSP at concentrations higher than 10 μM has been shown to reduce basal as well as VIP-stimulated cAMP levels in normal rat intestinal mucosal cells [4]. We therefore tested possible

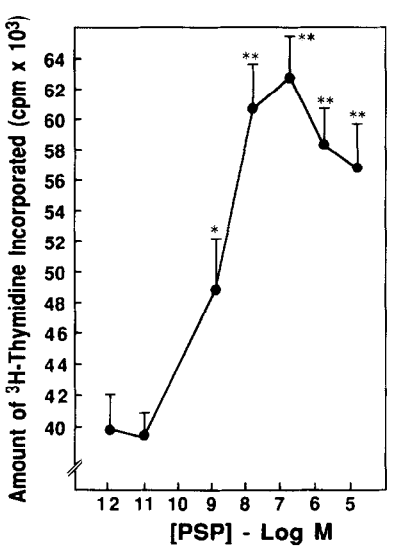

Fig.2. Increased [^{3}H]thymidine incorporation in breast carcinoma cells by PSP. MCF-7 cells were plated at 2 × 10^4 cells/well in 10% FBS-supplemented McCoy's 5A medium. After 3 days when cells were nearly confluent, the growth medium was changed to McCoy's 5A medium (no serum). After 24 h starvation cells were exposed to PSP at the indicated concentrations in fresh McCoy's 5A medium. Cells were harvested 24 h later. 4 h prior to the harvest 0.5 μCi [^{3}H]thymidine/well was added. Mean ± SD of triplicate wells are given. Amount of [^{3}H]thymidine incorporated by control cultures was 39.7 × 10^3 ± 1.2 × 10^3 cpm. Experiments were repeated three times with similar results.

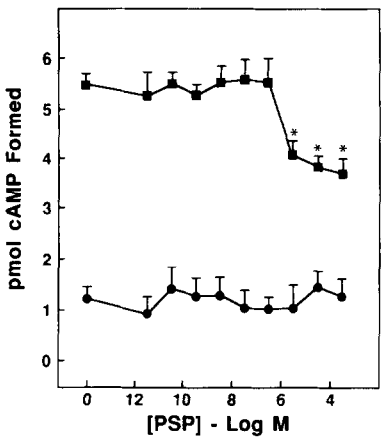

Fig.3. Effect of PSP and VIP on cAMP levels in HCT 116 cells. Levels of cAMP after incubation with PSP alone (●) or PSP with 0.3 μM VIP (■). Mean ± SD of three separate experiments are indicated. Levels of cAMP shown are amount of cAMP formed/6 × 10^5 cells/15 min (* significantly different from 0.3 μM VIP stimulated cAMP level).

modulation of cAMP levels in colon tumor cells by PSP (fig. 3). There was no significant effect on basal cAMP levels up to 300 μM PSP. VIP increases cAMP levels in a number of colon tumor cell lines [16,17]. As seen in fig. 4, 0.3 μM VIP caused a 5-6-fold increase in cAMP levels in HCT 116 cells. PSP at concentrations higher than 1 μM significantly reduced the VIP-mediated increase, in agreement with results obtained with normal intestinal cells [4]. This effect of PSP is probably not due to inhibition of VIP binding to its cell surface receptors since PSP did not block ^{125}I-VIP binding to normal intestinal cell membranes [4]. Also, VIP did not affect receptor binding of ^{125}I-PSP [4].

4. DISCUSSION

This is the first report, to our knowledge, of growth stimulatory effects of PSP. The colon tumor cell line (HCT 116) as well as the breast cancer cell line (MCF-7) responded to PSP with increased [^{3}H]thymidine incorporation. In both cell lines a maximal increase in [^{3}H]thymidine incorporation of 50–60% was observed, the half-maximal concentration being 500 nM for HCT 116 cells and lower (5 nM) for MCF-7 cells. Both these EC_{50} values are close to the IC_{50} value of 100 nM for ^{125}I-PSP binding to normal rat intestinal mucosal cells [4,5].

The proliferation of colon tumor cells has been shown to be inhibited by agents that enhance intracellular cAMP levels such as dibutyryl cAMP, forskolin, theophylline and VIP [17]. Also, the cAMP content of human colon carcinomas has been shown to be lower than that of the adjacent normal mucosa [18]. Therefore, the suppression of VIP-stimulated cAMP levels by PSP in HCT 116 cells may bear a causal relationship with stimulation of cell proliferation. Experiments designed to determine synthesis of PSP-like peptides by colon tumor cells need to be done in order to determine a possible role for such compounds in the etiology of colon cancer. In this regard it is of interest that the pS2 protein which displays a high degree of homology to PSP was recently shown to be expressed and secreted by normal human stomach cells, but not by several other normal tissues examined including colon [12].

The lack of availability of the pS2 protein has hampered progress toward elucidation of its physiological function [12]. The growth stimulatory effect of PSP on MCF-7 cells reported herein could very likely be due to cross-reaction with the pS2 receptor. Further studies with PSP may therefore help in understanding the role of pS2 in the mechanism of action of estrogen in breast cancer.

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