IN VITRO CHARACTERIZATION OF BOMBESIN AND CALCITONIN ON THE PROLIFERATION OF PC3, DU 145 AND LNCaP CANCER PROSTATIC CELL LINES.

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INTRODUCTION

The growth of normal and tumoral, benign and malignant prostate tissue is under the control of various hormones and/or growth factors. Many of these hormones and/or growth factors-mediated effects on the growth of human prostate cancer tissue are characterized by means of in vitro models, amongst which the most often used are LNCaP, DU 145, PC-3 cell lines. (1)(2)

The proliferation rate in LNCaP cell line is modulated by steroid -mediated influences. The DU 145 is often reported in the literature as being androgen irresponsive and finally the PC-3 cell line is usually considered as being the least hormone sensitive of the three models used. In addition to the steroid hormone mediated influences, the proliferation rate in the prostatic tumoral cell lines described can also be modulated by distinct growth factors, actually under investigation, which include certain neuropeptides. In this sense normal and malignat prostate contain neuroendocrine cells, characterized by the presence of typical secretory granules. These granules contain a variety of neuroendocrine peptides, including calcitonine (CT), bombesin, TSH like and somatostatin. (3) The presence of neuroendocrine cells in prostatic carcinoma has been correlated with tumor progression and poor prognostic. By other hand, the most prostate cancer initially respond to antiandrogenic therapy and the evolution to antiandrogenic-insensitive state almost inevitably leads to death from progressive disease. For this reason, it was suggested that neuroendocrine peptides could have effects on the behavior of prostate cancer cells (4)

The aim of the present study is to investigate the effect of two representative prostatic neuroendocrine cell peptides, in several well characterized human prostatic cancer cell lines (hormone sensitive and androgen irresponsive), to determine whether these peptides consistently stimulate cell proliferation.

MATERIAL AND METHODS

The prostatic carcinoma cell lines were obtained from American Type Culture Collection (LNCaP, DU-145) and Nuclear Iberica (PC-3). The cell lines were cultured in our laboratory at 37º under humidified atmosphere with 95% air and 5% CO_2 in microtiter plates (tissue culture grade, 96 wells, flat bottom. Labsystem), containing culture media , RPMI 1640 (ICN-FLOW), for LNCaP or DMEM (FLOW Lab), for DU 145 and PC-3, both supplemented with 4% penicilin-streptomicin (FLOW Lab) 2,5% FCS (SERVA), 0,4% gentamicin (GIBCO). Six groups were stablished in PC3 and DU145 : control, bombesin 1nm/ml, bombesin 5 nm/ml, bombesin 10 nm/ml, calcitonin 50 pg/ml, calcitonin 500 pg/ml, and ten in LNCaP, six treated with neuropeptides as described before, a treatment group with DHT 1,35 nm/well alone, and five more treated with neuropeptides plus DHT at the same doses .

Cell proliferation was assessed by means of the colorimetric XTT assay, as previosly described (5). Briefly, after incubation of cells with different treatments, XTT solution (Boehringer Mannheim) was added to each well in a final concentration of 0.3 mg/ml . The assay is based on living cells reduction of the yellow product of formazan to form a blue product that is water soluble. Thus, the intensity of blue staining is proportional to the number of cells alive at the moment of analysis. Measurements were made at 24, 48, 72 hours of culture. We also compared the data obtained by direct cell counting by hematocytometer.

RESULTS

The effects of bombesin and calcitonin on cell proliferation of prostate cancer cell lines are shown in figures 1,2,3,4.(AU=Absorbance units). At 1nM, 5nM, and 10 nM concentration of bombesin, under 2,5% serum supplementation, the neuropeptide caused a maximal stimulation, in PC-3 cell proliferation, at 72 hours. In the DU 145 cell line the optical densities values (OD) remained almost unchanged always above control at 24, 48 and 72 hours. In both cell lines the cell proliferation ocurrs in a dose independent manner. The OD in LNCaP cell line with bombesin were always under the mean values of DHT stimulation of cell proliferation. Furthermore, the cell proliferation in LNCaP was no affected by bombesin plus DHT supplementation. The addition of 50 pg/ml and 500 pg/ml of calcitonin to PC-3 and DU 145 cell lines show an OD consistent with cellular proliferation in a dose dependent manner. In LNCaP cell line calcitonin OD values are not consistent with cell proliferation.

DISCUSSION

The human prostatic gland contains numerous neuroendocrine peptides located either in nerves or in prostatic neuroendocrine cells. While the presence of these peptides in benign and malignant prostate tissue has been extensively documented, less is known about their biological activity. It has been suggested that neuroendocrine peptides may have biological effects on prostate cancer cells.(6)(4)

Our data show that the growth of PC-3 and DU 145 cell lines, in 2,5% FCS supplemented medium, is stimulated by bombesin and calcitonin. Double labelling methods for chrA and the proliferation associated MIB-1 antigen indicate that endocrine -paracrine cells may be involved in the control of cell proliferation trough a paracrine mechanism (7). Bombesin and GRP appear to act as autocrine or paracrine growth factor in a variety of cells (8). These studies indicate that bombesin/GRP are involved in the regulation of cell proliferation and differentiation. In the other hand, it has been described that DU 145 cell line showed an increase in cAMP response to calcitonin receptor m RNA. (4) To our knowledge, this is the first report that describe a proliferative action of bombesin in DU 145 and calcitonin in PC-3, both irresponsive androgen cell lines. In contrast , in the present study the magnitude of the reponse to bombesin and calcitonin in LNCaP cells - an androgen sensitive human prostatic cell line-, was relatively similar to control in comparision with PC-3 and DU 145 cells. Opposite, Shah described (9) a dose dependent increase in ^3H thymidine

incorporation in these cells. However, recently, LNCap cells did not demostrate large c AMP response to calcitonin and they lacked calcitonin receptor mRNA (4). LNCaP cells share features with normal prostate epithelial cells including the presence of androgen receptors and synthesis of prostate-specific antigen. (10) Neuroendocrine differentiation ocurrs to a variable degree in virtuallly all prostatic adenocarcinomas, and may correlate with a high histological grade and poor prognosis (11). The discovery of bombesin/ GRP like peptides functioning as autocrine growth factors in human small cell lung carcinoma stimulated the development of several classes of bombesin/ GRP antagonists (12).This aspect should be explored in order to improve therapy for endocrine-irresponsive tumors, which represent a large number of prostate cancer in men.

CONCLUSIONS

The results reported in the present study indicate that the bombesin and calcitonin are potent mitogens "in vitro" and may be potential paracrine growth promoters in stablished androgen irresponsive human prostatic carcinoma cells.

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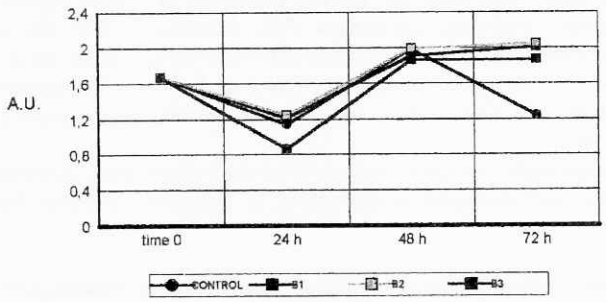
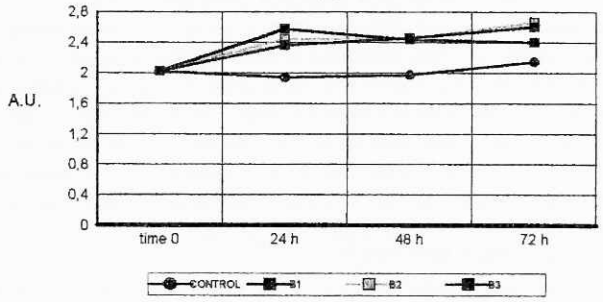
PC - 3 / BOMBESIN

figure 1



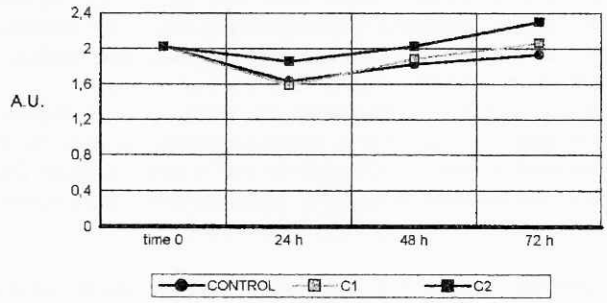
Du145 / BOMBESIN

figure 2



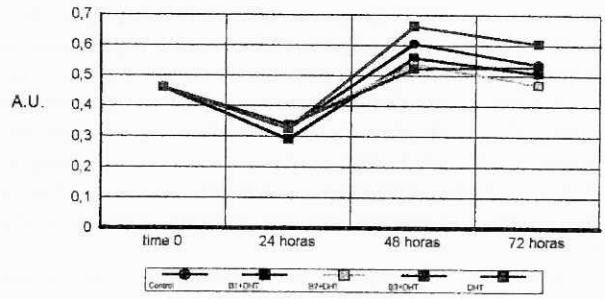
Du145 / CALCITONIN

figure 3



LNCaP BOMBESIN+ DHT

figure 4



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