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Short communication

Noradrenaline inhibits the programmed cell death induced by 1,25-dihydroxyvitamin D_3 in glioma

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Abstract

The rat glioma cell line C6.9 has been recently reported to respond to 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) by the induction of a programmed cell death. Since, in vivo, glial cells are thought to be exposed to several neurotransmitters, we investigated the possibility of a neurotransmitter-mediated inhibition of this active cell death process. Noradrenaline and the β-adrenoceptor agonist isoproterenol showed significant inhibition of the $1,25(OH)_2D_3$-induced programmed cell death. The β-adrenoceptor antagonist propanolol reversed this inhibition, while the α-adrenoceptor antagonist yohimbin was devoid of any effect. This suggests that the efficiency of antiproliferative vitamin D-related therapies could be influenced by endogenous levels of noradrenaline.

Keywords: Vitamin D; Glioma; Apoptosis; Noradrenaline

1. Introduction

Over the past few years accumulating evidence has suggested the involvement of programmed cell death in the elimination of some tumor cells. This programmed cell death can occur naturally through immune defense mechanisms and tissue-specific homeostatic controls, or it can be induced artificially by chemotherapeutic agents such as drugs and radiation (Schwartzman and Cidlowski, 1993).

Glioma are the most common type of primary brain tumor, and are responsible for approximately 2.5% of all cancer deaths. The relative ineffectiveness of surgery, radiation therapy and chemotherapy in the control of the evolution of glioma has contributed to the poor prognosis of this disease. The molecular mechanisms involved in the development of this malignancy appear complex. Loss or mutation of the p53 tumor suppressor gene as well as amplification and/or overexpression of platelet-derived growth factor and epidermal growth factor receptors have been reported (Fleming et al., 1992). Successive genetic changes have also been associated with an increased malignancy of glioma cells. However, the existence of a common oncogenic pathway leading to the formation of all glioma seems unlikely.

1,25-Dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) is a seco-steroid hormone which might, in addition to its well known action on calcium homeostasis, play a role in the nervous system. This involvement in the nervous system is supported by the existence of binding sites for $1,25(OH)_2D_3$ in several structures of the brain (Stumpf and O'Brien, 1987), by the synthesis of $1,25(OH)_2D_3$ by activated microglial cells (Neveu et al., 1994a), and by findings that the hormone regulates the expression of several genes encoding neurotrophins, such as the nerve growth factor, neurotrophin-3 and neurotrophin-4, as well as the low-affinity nerve growth factor receptor (Wion et al., 1991; Neveu et al., 1994b; Naveilhan et al., 1996). $1,25(OH)_2D_3$ has also been shown to modulate cell growth and differentiation both in vitro and in vivo in a variety of tumor model systems (DeLuca and Ostrem, 1986). Most of these actions are mediated via the nuclear vitamin D receptor which belongs to the steroid/thyroid/retinoic acids receptor superfamily, and which can form heterodimers with retinoic acids and thyroid hormone receptors (Studzinski et al., 1993).

We recently reported that a rat glioma cell line, C6.9, derived from rat C6 glioma cells, responds to $1,25(OH)_2D_3$ treatment by inducing a cell death program (Baudet et al., 1996a,b). C6.9 cell death typically includes DNA fragmen-

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tation and the induction of several genes including *cmyc*, *p53* and *gadd45* (Naveilhan et al., 1994; Baudet et al., 1996a,b). These findings have led to the suggestion that $1,25(OH)_2D_3$, or its low calcemic analogs, could be of potential interest in the treatment of brain glial tumors (Naveilhan et al., 1994; Baudet et al., 1996a).

The possible involvement of glia-neuron interactions in the genesis of glioma and in their resistance to therapeutic intervention has until now received little attention. Nevertheless, the role of secreted substances which mediate paracrine interactions during oncogenesis is now clearly established. In the case of glioma, in addition to autocrine loops involving factors such as platelet-derived growth factor or epidermal growth factor (Fleming et al., 1992), these substances could be factors secreted from neurons into the neuron-astroglia synaptic microenvironment. Since programmed cell death is theoretically amenable to modulation by exogenous agents, we investigated the possible interference of neurotransmitters with the active cell death induced by $1,25(OH)_2D_3$ in C6.9 glioma cells.

2. Materials and methods

Rat C6.9 glioma cells (Baudet et al., 1996b) were cultured in F12 medium supplemented with 10% fetal calf serum and used between passages 5 and 20. The day before the addition of $1,25(OH)_2D_3$, cell culture medium was replaced with a serum-free medium consisting of F12 medium supplemented with insulin (5 μg/ml), transferrin (5 μg/ml), selenium (5 ng/ml), penicillin (100 units/ml), streptomycin (100 μg/ml) and amphotericin B (0.25 μg/ml). After 24 h of treatment with 10^{-7} M $1,25(OH)_2D_3$ the medium was changed and cells were cultured in fresh serum-free medium depleted of $1,25(OH)_2D_3$, but supplemented with or without the different neurotransmitters. This medium was renewed each day. After 6 days in culture, cell viability was quantified using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay (Jordan et al., 1992). For detection of DNA fragmentation, pelleted cells were resuspended in 50 mM Tris-HCl (pH 8.0), 50 mM EDTA, 0.5% (w/v) sodium lauryl sarkosinate and 0.5 mg/ml proteinase K. After an incubation of 1 h at 55°C the lysate was treated for an additional 1 h with DNase-free RNase at 50°C. Then the samples were mixed with 1% low-gelling temperature agarose at 62°C and fractionated onto a 1.5% agarose gel (Baudet et al., 1996a).

3. Results

To investigate the existence of possible interactions between neurotransmitters and the cell death program induced by $1,25(OH)_2D_3$ in C6.9 glioma cells, C6.9 cells treated for 24 h with 10^{-7} M $1,25(OH)_2D_3$ were cultured in the presence of 10^{-5} M noradrenaline. This catecholamine was used since the existence of functional β-adrenoceptors has been detected on C6 cells (Oey, 1975). Accordingly with previous results, death occurred in glioma cells 6 days after a treatment for 24 h with $1,25(OH)_2D_3$. However, results presented in Fig. 1 demonstrate that this cell death program is suppressed when cells are cultured in the presence of the neurotransmitter noradrenaline, while histamine (10^{-5} M) is devoid of any effect. This cell death inhibition was also observed with isoproterenol, a β-adren-

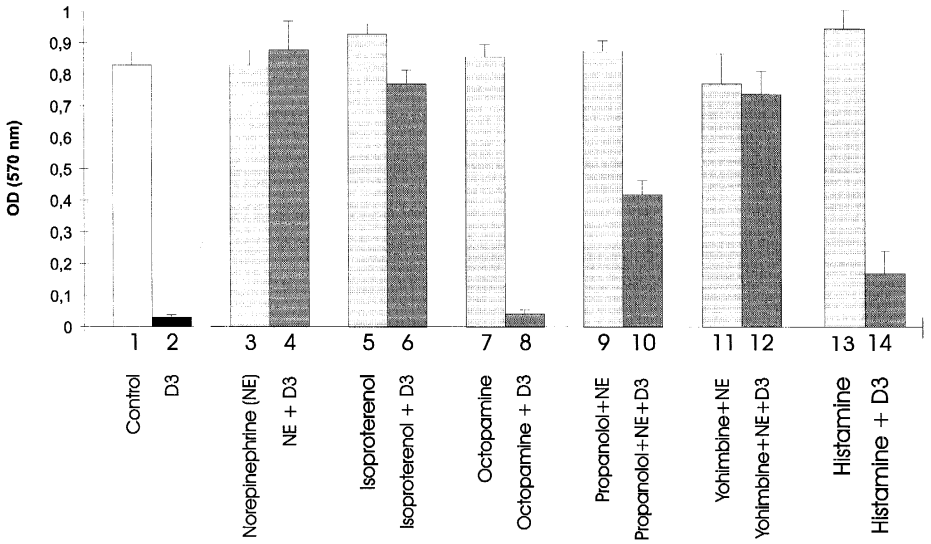

Fig. 1. Comparison of the effects of noradrenaline, histamine and different α- and β-adrenoceptor agonists and antagonists on the inhibition of the $1,25(OH)_2D_3$-induced cell death. $1,25(OH)_2D_3$ was added at day 0 for 24 h. Then 10^{-5} M of the different neurotransmitters, agonists or antagonists were added and the MTT assay was performed at day 6. All neurotransmitters, agonists and antagonists are added at a concentration of 10^{-5} M. Each point represents the mean ± S.E.M. of a triplicate sample.

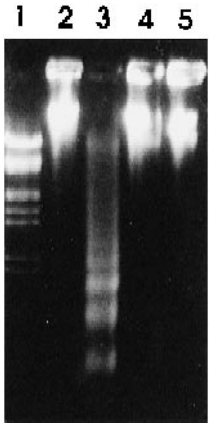

Fig. 2. Inhibition of internucleosomal DNA fragmentation by noradrenaline in C6.9 cells treated with $1,25(OH)_2D_3$. DNA was extracted at day 6 from C6.9 cells and the formation of oligonucleosomal fragments was determined by agarose gel electrophoresis. Lane 1, molecular mass markers; lane 2, DNA from control cells; lane 3, DNA from cells treated for 24 h with 10^{-7} M $1,25(OH)_2D_3$ and then cultured for 5 days in serum-free medium; lane 4, DNA from cells treated for 5 days with 10^{-5} M noradrenaline; lane 5, DNA from cells treated for 24 h with 10^{-7} M $1,25(OH)_2D_3$ and then for 5 days with 10^{-5} M noradrenaline.

oceptor agonist, but not with octopamine, a specific α-adrenoceptor agonist. In addition, propranolol, a β-adrenoceptor antagonist, reduces by 50% the protective effect observed with noradrenaline, while the α-blocker, yohimbine, is devoid of any effect. Supraphysiological concentrations of noradrenaline were used in these experiments because this compound is rapidly oxidised in tissue culture medium. Accordingly, even if the concentrations of antagonists used in this study are consistent with the concentrations used by other investigators (Buonassi and Venter, 1976), we cannot exclude the possibility that partial or failing activity with some blockers may be due to suboptimal concentrations. Thus, taken together these results suggest that the protective effect observed with noradrenaline is mediated through interactions of noradrenaline with β-adrenoceptors. The observation that addition of 8BrcAMP to the culture medium of $1,25(OH)_2D_3$-treated cells also suppresses this cell death program (data not shown), further supports the involvement of the β-adrenoceptor-adenylate cyclase-phosphodiesterase system in this process. Finally, results presented in Fig. 2 showed that noradrenaline protects the $1,25(OH)_2D_3$-treated cells from DNA fragmentation.

4. Discussion

The finding reported here, that noradrenaline can suppress a programmed cell death induced by $1,25(OH)_2D_3$, could potentially have both physiological and pharmacological implications. Thus it has been proposed that the capacity of activated microglial cells to produce $1,25(OH)_2D_3$ could be part of the mechanisms limiting the extension of some glioma in vivo (Baudet et al., 1996b). Results presented here suggest that if a such mechanism exists it might be inoperative in the close vicinity of noradrenergic neurons. In addition, in view of the possible role of $1,25(OH)_2D_3$ analogs in the treatment of proliferative diseases, it could be important to determine whether the cross-talk observed here in glioma between noradrenaline and the $1,25(OH)_2D_3$-induced programmed cell death is also observed in other cancer cells, such as breast, colonic, and prostate carcinoma cells, for which an antiproliferative action of $1,25(OH)_2D_3$ has been demonstrated (DeLuca and Ostrem, 1986).

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