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# Actinomycin D decreases protein kinase C content and induces neuritogenesis in neuroblastoma cells

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We have previously reported that inhibition of protein kinase C induces differentiation of neuroblastoma cells in culture. It is shown now that actinomycin D, a well known inhibitor of DNA synthesis, reduces selectively the content of protein kinase C and induces neuritogenesis in Neuro 2a cells in culture.

Neuritogenesis; Protein kinase C; Actinomycin D; Neuroblastoma

## 1. INTRODUCTION

We have previously shown that inhibition of protein kinase C induces differentiation of neuroblastoma cells in culture. This was associated with inhibition of cell proliferation and of DNA synthesis [1–3]. To clarify the mechanism by which protein kinase C regulates cellular differentiation/proliferation, we have assessed the effects of actinomycin D, a well known inhibitor of DNA synthesis. We show here that actinomycin D decreases protein kinase C and induces neuritogenesis in neuroblastoma cells in culture.

## 2. MATERIALS AND METHODS

The clonal cell line neuro 2a (C1300 mouse neuroblastoma) was obtained from the American Type Culture Collection. Cells were grown in Eagle's minimum essential medium (MEM) supplemented with 10% heat-inactivated fetal bovine serum, 100 IU/ml of penicillin, and 100 μg/ml of streptomycin. Actinomycin D was dissolved in methanol and dilutions were made in MEM. Cells were seeded at 200 000 /ml. To quantify neuritogenesis several randomly chosen fields were photographed in a phase-contrast light microscope and neurites were counted in 500–1000 cells. The viability of cells was determined by trypan blue exclusion. Electrophoresis and immunoblotting were carried out as in [3].

## 3 RESULTS AND DISCUSSION

As shown in Fig. 1, actinomycin D induces neuritogenesis in neuroblastoma cells in culture. The percentage of differentiated cells increased with the dose of actinomycin D (Fig. 2). The inhibitory effect of actinomycin D on DNA synthesis is also shown in Fig. 2. Both induction of neuritogenesis and inhibition of DNA synthesis were near maximal at ≈ 0.4 μg/ml actinomycin D. Under the conditions used cell viability was not significantly affected. Cell viability was 86% for control cells and 85% and 78% for cells incubated

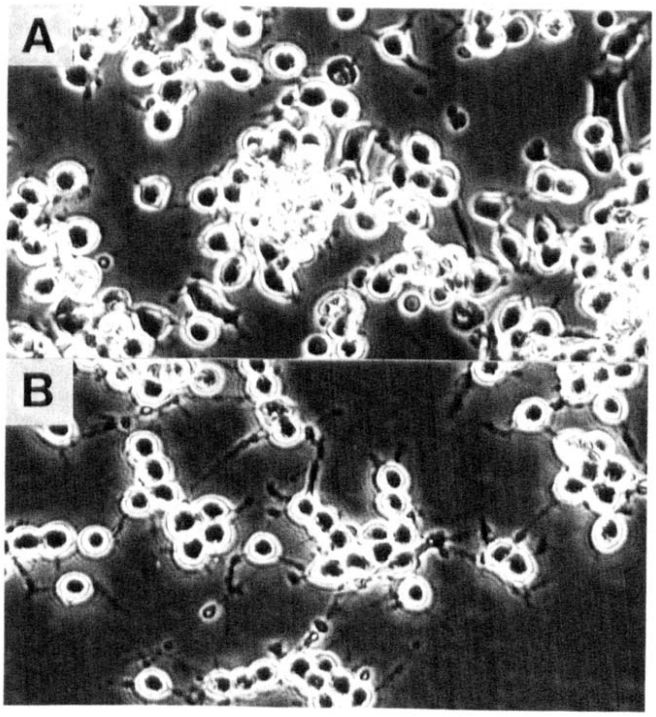


Fig. 1. Induction of neuritogenesis in neuro 2a cells by actinomycin D. Cells were incubated in the absence (A) or the presense (B) of 0.5 μg/ml actinomycin D, for 24 h and photographed using a phase-contrast light microscope.

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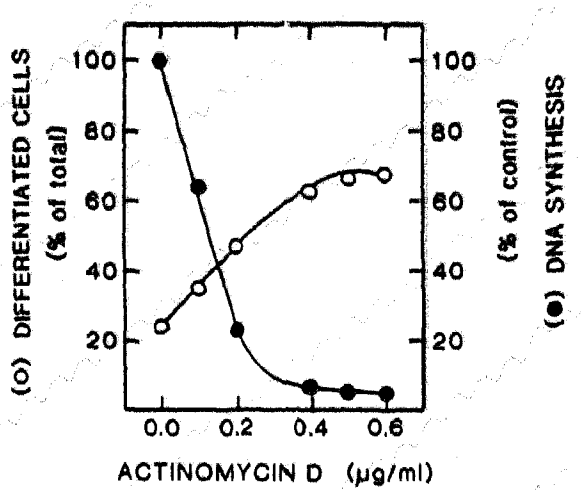


Fig. 2. Effect of actinomycin D on DNA synthesis and cell differentiation in neuro 2a cells. Cells were seeded at 100000 /ml. After 24 h of subculture actinomycin D was added; 23 h later 5 μCi/ml ([$^3$H]methyl)thymidine (5 Ci/mmol) was added and synthesis of DNA was determined after 1 h by measuring trichloroacetic acid insoluble radioactivity. Other sets of cells were cultured under the same conditions and, 24 h after addition of actinomycin D, several were randomly chosen and photographed using a phase-contrast light microscope. The percentage of cells possessing neurites is given.

for 24 h in the presence of 0.2 and 0.5 μg/ml actinomycin D, respectively.

It has been previously shown that inhibition of protein kinase C induces neuritogenesis in neuro 2a cells. We therefore tested, by immunoblotting, if incubation of these cells with actinomycin D affects protein kinase C content. As shown in Fig. 3, after 6 h of incubation with 0.5 μg/ml of actinomycin D, the protein kinase C content in neuro 2a cells was significantly reduced (39% as determined by measuring the intensity of the spots in a laser densitometer). Under these conditions 51% of the cells were differentiated.

The loss of protein kinase C in cells treated with actinomycin D seems to be selective, since the pattern of proteins is affected only very slightly. As shown in Fig. 4, the only marked change is the accumulation of a protein with an $M_r \approx 65\,000$; however, this protein is not accumulated in cells incubated with 500 μM H7, an inhibitor of protein kinase C, which also induces differentiation. It seems, therefore, that induction of this protein does not play an important role in the process of differentiation.

The above results clearly show that actinomycin D induces neuritogenesis in neuroblastoma cells in culture and that this was associated with a decrease in the content of protein kinase C. These findings agree with previous results showing that inhibition of protein kinase C by H7 induces differentiation of neuro 2a cells.

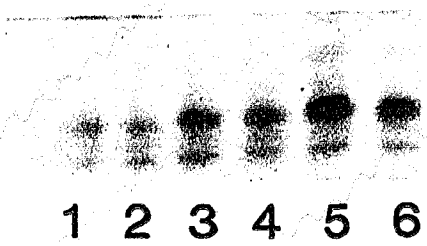


Fig. 3. Effect of actinomycin D on protein kinase C content in neuro 2a cells. Cells were seeded at 200000 /ml and 24 h later actinomycin D was added to reach 0.5 μg/ml. After 6 h cells were washed with PBS (1.5 mM potassium phosphate, 0.8 mM sodium phosphate, 137 mM NaCl, 2.7 mM KCl, pH 7.4), scraped in PBS containing 25 μg/ml aprotinin, and collected by centrifugation. The pellet was resuspended in 62.5 mM Tris-HCl, pH 6.8, containing 10% glycerol, 5% 2-mercaptoethanol, and 2.3% SDS. Samples containing 20 (lanes 1 and 2), 40 (lanes 3 and 4) or 60 (lanes 5 and 6) μg of protein were subjected to electrophoresis and immunoblotting as in [4] using a monoclonal antibody against protein kinase C (Amersham) that recognizes α- and β-isoforms.
*Odd lanes*: control cells; *even lanes*: cells treated with actinomycin D.

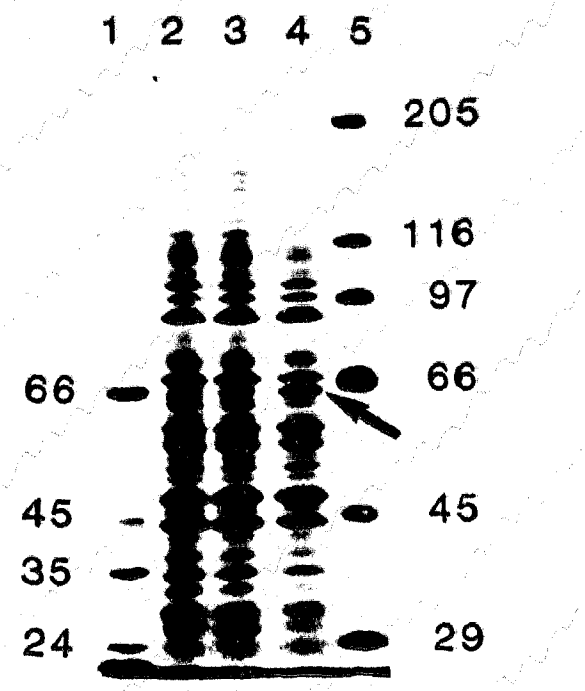


Fig. 4. Effect of actinomycin D on the pattern of proteins of neuro 2a cells. Cells were treated as in Fig. 3 except that incubations were for 24 h. Incubations were with 0.5 μg/ml actinomycin D (lane 4) and with 500 μM H7 (lane 2). *Lane 3*: control cells; *Lanes 1 and 5*: standards having the indicated $M_r \times 10^{-3}$

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