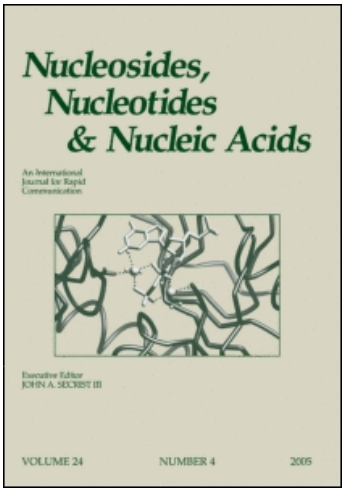

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Cyclic Nucleotides and Neuroblastoma Differentiation

E. Messina[a]; F. Lupi[a]; L. Barile[a]; A. Giacomello[a]
[a] Department of Experimental Medicine and Pathology, "La Sapienza" University, Rome, Italy

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Cyclic Nucleotides and Neuroblastoma Differentiation

E. Messina,* F. Lupi, L. Barile, and A. Giacomello

Department of Experimental Medicine and Pathology,
''La Sapienza'' University, Rome, Italy

ABSTRACT

We have shown that intracellular cGMP levels increase during retinoic acid- and mycophenolic acid-induced neuroblastoma differentiation and that a 6 days treatment with 1 mM dbcGMP lead LAN5 cell to elaborate a network of neuritic processes suggesting an involvement of cGMP in neuroblastoma differentiation. We have also investigated the effects of some specific inhibitors of phosphodiesterases (PDE1, PDE3, PDE4 and PDE5) on human neuroblastoma (LAN5 and SHEP) growth and differentiation. After six days of incubation in the presence of each specific inhibitor at $10 \times IC_{50}$ levels a cytostatic and differentiating effect was only observed with the PDE5 inhibitors Zaprinast and MY-5445. The cytostatic effect of these compounds increased increasing their concentrations far above their IC_{50} levels for PDE5, suggesting that these compounds could act by interfering with other molecular events than direct cGMP-PDE inhibition. No appreciable effect was observed using Dipyridamole, another specific PDE5 inhibitor.

Key Words: cAMP; cGMP; PDEs inhibitors; Neuroblastoma proliferation and differentiation.

*Correspondence: E. Messina, Department of Experimental Medicine and Pathology, ''La Sapienza'' University, Rome, Italy.

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INTRODUCTION

Cyclic AMP (cAMP) and cyclic GMP (cGMP) act as second messengers in a multitude of cellular processes including proliferation and differentiation. Elevation of cAMP is sufficient for inhibiting proliferation and promoting neurite outgrowth from neuroblastoma cells.[1,2] Although intracellular cGMP levels increase during retinoic acid and mycophenolic acid-induced neuroblasoma differentiation, the role of this cyclic nucleotide is less clear.

MATERIALS AND METHODS

Exponentially growing LAN5 and SHEP human neuroblastoma cells were treated with dbcAMP and dbcGMP (from 0 to 1 mM) or with PDE1, PDE3, PDE4, PDE5 specific inhibitors (from 0 to 10 × IC50 levels) for 0 to 6 days. The specific PDE5 inhibitors Zaprinast and MY-5445 were also employed at higher concentrations (up to around 100 × IC_{50}). Cell proliferation and viability was quantified using the cell proliferation reagent WST-1. Cell differentiation was assessed by morphological changes. Intracellular levels of cyclic nucleotides were determined using enzyme-immunoassay systems.

RESULTS AND DISCUSSION

We have shown that LAN5 human neuroblastoma cells elaborated a network of neuritic processes during a 6 days treatment with 1 mM dbcGMP, with a neurite outgrowth similar to that obtained using 1 mM dbcAMP.

The intracellular levels of cAMP and cGMP are determined by a balance between formation by adenylate and guanylate cyclases, respectively, and degradation by phosphodiesterases (PDEs). We have observed a very high increase of guanylate cyclase activity during MPA induced differentiaton of LAN5 cells. PDEs have been classified into 11 families with multiple isoforms within each family.[3] PDE4 (cAMP specific) and PDE5 (cGMP specific) activities are induced in neuroblastoma lines under differentiating conditions.[4] Isoenzymes of the Ca2 + -calmodulin dependent PDE1 family are highly represented in different regions of the nervous system.[3] PDE3 isoforms (cGMP inhibited) may be expressed in cells of neuroectodermal origin.[5]

In the present study we have investigated the effects of the specific inhibitors of PDE1 (MMPX), PDE3 (Cilostamide, Quazinone, Milrinone, Zardaverine), PDE4 (Rolipram, Etazolate, Zardaverine), PDE5 (Zaprinast, MY-5445, Dipyridamole) on neuroblastoma growth and differentiation. LAN5 and SHEP human neuroblastoma cells were treated for 3 to 6 days with each specific inhibitor at concentrations ranging from 0 to 10 × IC_{50} μM. Spectrophotometric quantification of cell proliferation and viability showed a decreased cell proliferation only in response to Zaprinast and MY-5445. As shown in the Fig. 1 the cytostatic effect of these compounds increased increasing their concentrations far above their IC_{50} levels for PDE5 (0.8 μM for Zaprinast and 0.6 μM for MY-5445).

After 6 days of incubation of both cell lines in the presence of these two PDE5 inhibitors at 10 × IC_{50} concentrations an increase in neurite outgrowth was observed.

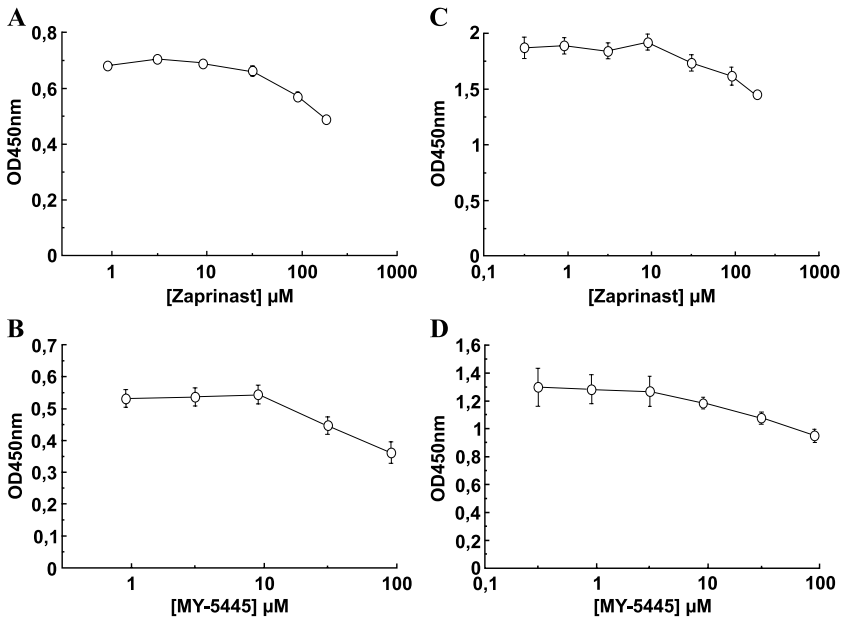

Figure 1. Cytostatic effects of Zaprinast and of MY-5445 on LAN5 (A and B) and SHEP (C and D) human neuroblastoma cell lines. Exponentially growing cells were incubated with the PDE5 inhibitor for 3 (B and D) or 6 (A and C) days. Cell proliferation reagent WST-1 was added and cells were reincubated for another 3 h. Thereafter, the formazan formed was quantified at 450 nm with an ELISA plate reader. Each value is the mean ± SD of three experiments.

Preliminary experiments showed that at the $10 \times IC_{50}$ drug concentrations of specific PDE inhibitors intracellular cGMP levels obtained with Zaprinast or MY-5445 were comparable to those obtained with Dipyridamole, a PDE5 inhibitor without significant effects on neuroblastoma growth and differentiation. This last result and the increase of the cytostatic effect at concentrations of the PDE5 inhibitors higher than 10 folds their IC_{50} values suggest that Zaprinast and MY-5445 could act by interfering with other molecular events than direct cGMP-PDE inhibition.

In conclusion we have demonstrated an involvement of cGMP in neuroblastoma differentiation and a significant antiproliferative and differentiating action of Zaprinast and MY-5445. These observations suggest that these PDE5 inhibitors could have a potential role in the management of neuroblastoma patients.

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