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Inhibition of protein kinase C induces differentiation of neuroblastoma cells

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It is shown that 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H7), a specific inhibitor of protein kinase C, induces neuritogenesis in neuro 2a cells. The percentage of differentiated cells was 9%, 20%, 59% and 85% at 0, 17, 85 and 500 μM H7, respectively. The number of neurites/cell increased 2-, 8- and 14-fold over the controls for 17, 85 and 500 μM H7, respectively. These results indicate that protein kinase C plays a key role in the control of differentiation of neural cells and that its specific inhibition may be of basic as well as of practical importance.

Protein kinase C; Isoquinolinylsulfonyl)-2-methylpiperazine, 1-(5-; Neural differentiation; Neuritogenesis

1. INTRODUCTION

Gangliosides are known to induce the differentiation of neuroblastoma cell lines with concomitant sprouting and extension of neurites [1,2], although the underlying molecular mechanism remains unknown [3,4]. Since others have shown that gangliosides are inhibitors of protein kinase C [5,6], it seemed possible that the two findings were related and that gangliosides might stimulate neuritogenesis by inhibiting the activity of protein kinase C. To check this hypothesis, we have tested the effect of 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H7), a specific inhibitor of protein kinase C [7], on neurite outgrowth of neuro 2a cells. We found, and show here, that H7 is a potent inducer of neuritogenesis, indicating a key role for protein kinase C in the control of differentiation of neural cells.

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2. MATERIALS AND METHODS

2.1. *Cell cultures*

The clonal line neuro 2a, C-1300 mouse neuroblastoma, was obtained from the American Type Culture Collection (Rockville, MD, USA). They were grown at 37°C in Eagle's minimum essential medium supplemented with 10% fetal bovine serum, 100 IU/ml penicillin and 100 μg/ml streptomycin. Cells wers subcultured twice weekly. Viability was assessed with Trypan blue.

2.2. *Incubations with H7*

H7 (34 mM) was dissolved in dimethylsulfoxide and added after 24 h of subculture. The same volume of dimethylsulfoxide was added to the controls. Medium was changed and fresh H7 was added every second day.

2.3. *Quantification of neuritogenesis*

Several randomly chosen fields of the cultures were photographed using a phase-contrast light microscope. The number of neurites on each cell was counted and their lengths were measured.

3. RESULTS AND DISCUSSION

As shown in fig.1, H7 markedly induced neuritogenesis in neuro 2a cells. To quantify neuritogenesis, the number of neurites/cell after 24 h of treatment with H7 was counted. As shown in table

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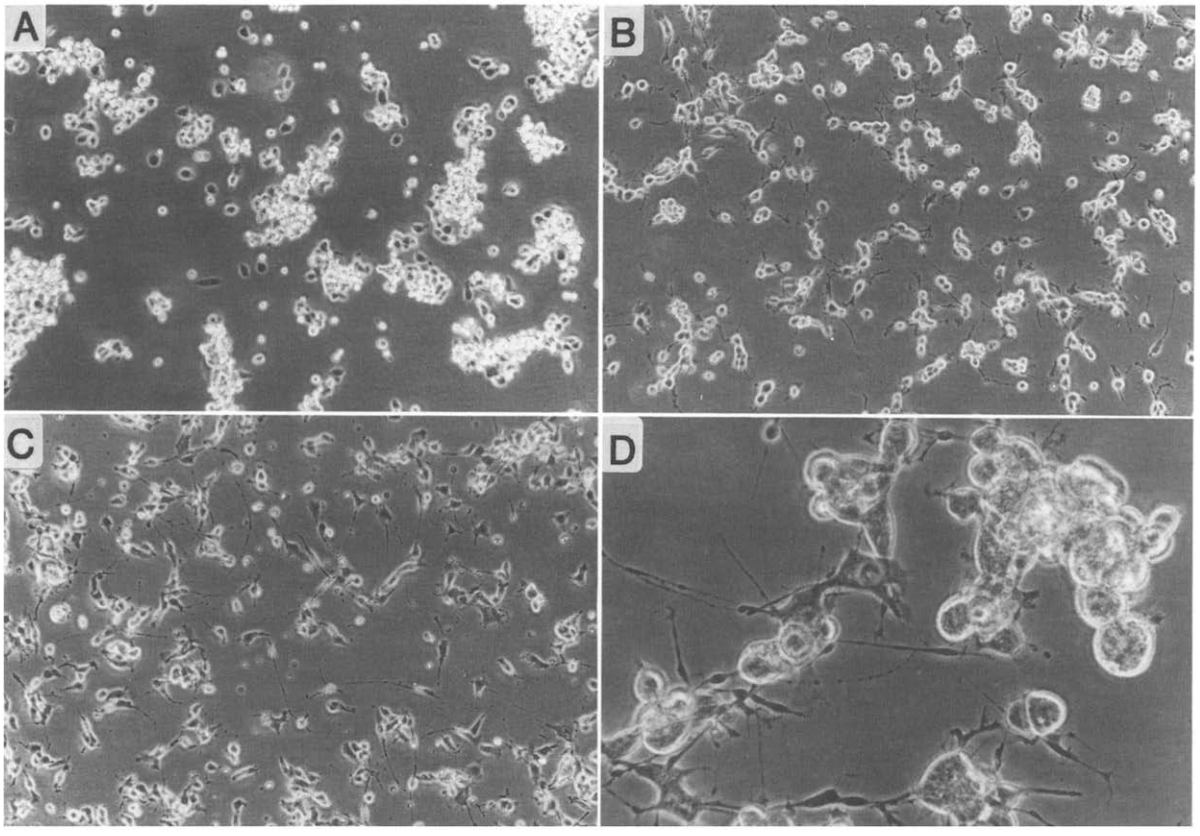

Fig.1. Effect of H7 on neuritogenesis in neuro 2a cells. Cells were incubated for 24 h in the absence (A) or the presence of 85 μM (B) or 500 μM H7 (C,D) and then photographed with a phase-contrast light microscope.

1, in controls, only 9% of cells are differentiated. The number of differentiated cells increased with the dose of H7 added, reaching 20%, 59% and 85% for 17, 85 and 500 μM H7, respectively. Also, the mean number of neurites/cell increased markedly with the concentration of H7, i.e. 2-, 8- and 14-fold higher than in controls for 17, 85 and 500 μM H7, respectively. At all doses of H7 used, neurites were approximately 3 times longer than in controls (table 1). As shown, up to 500 μM H7 can be used with minimal cell toxicity; at this concentration cell viability was 80%, while for controls it was 91%.

Table 1

Effect of H7 on neuritogenesis in neuro 2a cells

H7 (μM)	Viability (%)	Differentiated cells (% of total)	Neurites/cell	Mean length (μm)
0	91	9	0.14	23 ± 8
17	88	20	0.30	58 ± 14
85	86	59	1.14	60 ± 12
500	80	85	1.90	61 ± 14

Neuro 2a cells were seeded at 200 000/ml, H7 was added after 24 h and 24 h later viability was measured and several randomly chosen fields were photographed. The neurites per cell were counted and measured in at least 200 cells per group; control and each concentration of H7 used

The above results clearly demonstrate that H7, a specific inhibitor of protein kinase C, induces differentiation and neuritogenesis in neuro 2a cells. This indicates that protein kinase C is involved in the control of differentiation and neuritogenesis in neuro 2a cells.

It was shown several years ago that gangliosides induce differentiation and neuritogenesis in neuroblastoma cells [1,2], and later that they also inhibit protein kinase C [5,6]. The present findings in-

dicate that the effect of gangliosides on differentiation is likely due to its inhibitory effect on protein kinase C.

Gangliosides have been used successfully in animal models for the treatment of certain neurological disorders. The following beneficial effects have been reported for gangliosides: stimulation of the regeneration of the nervous system and facilitation of recovery after CNS damage [8], acceleration of reinnervation [9], facilitation of learning performance [10], decreased mortality after induction of global ischemia [11], counteraction of the slowing of nerve conduction in diabetic neuropathy [12], reduction of retrograde degeneration after neocortical lesions [13], protection against excitotoxin damage [14] and reduction of vincristine-induced neuropathy [15]. The findings reported here suggest that similar effects might be obtained by using specific inhibitors of protein kinase C.

In conclusion, the results we report here indicate that protein kinase C plays a key role in the control of neural differentiation.

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