

Mannose-6-phosphate stimulates proliferation of neuronal precursor cells

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The mitogenic signal function of mannose-6-phosphate (Man-6-P)/insulin-like growth factor II (IGF-II) receptors was studied in neuronal precursor cells from developing rat brain (E15). About 30% of the cellular Man-6-P/IGF-II receptors were present on the cell surface. Man-6-P and IGF-II stimulated DNA synthesis twofold and their effects were additive. Antibody 3637 to the Man-6-P/IGF-II receptor blocked the response to Man-6-P but not that to IGF-II. Other phosphorylated hexoses were also active. Fructose-1-phosphate was equally potent with Man-6-P, whereas glucose-6-phosphate was 5 times less potent. We conclude that Man-6-P-containing proteins and IGF-II act as mitogens in developing brain by interaction with the Man-6-P/IGF-II receptor and the IGF-I receptor, respectively.

Insulin-like growth factor; Mannose-6-phosphate; Receptor; Mitogenesis; Neuronal precursor cell

1. INTRODUCTION

Structural and biochemical evidence has shown that the mammalian cation-independent mannose-6-phosphate (Man-6-P) receptor is identical to the insulin-like growth factor II (IGF-II) receptor and that the two ligands bind simultaneously to different sites [1]. The main function of the Man-6-P receptor is to translocate phosphomannosylated lysosomal enzymes from the Golgi to lysosomes, but about 10–20% of Man-6-P/IGF-II receptors are present on the cell surface and mediate endocytosis of Man-6-P-containing ligands and IGF-II [1,2]. It is not clear whether the Man-6-P/IGF-II receptor has a role in intracellular signalling. The cellular effects of IGF-II are generally mediated by the insulin-like growth factor I (IGF-I) receptor, although the Man-6-P/IGF-II receptor may be also involved in IGF-II signal transduction in selected transformed cell lines [1,3]. Furthermore, Man-6-P stimulates expression of its receptor on the surface of fibroblasts by a mechanism involving G-proteins [4], and in a mammary cancer cell line, a secreted phosphomannosylated 52 kDa protein acts as an autocrine growth factor [5]. These observations suggest that Man-6-P-containing proteins induce cellular responses via the Man-6-P/IGF-II receptor. To evaluate this possibility, we have here studied the action of Man-6-P on DNA synthesis in neuronal precursor cells from fetal rat brain (E15).

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2. MATERIALS AND METHODS

2.1. Chemicals

Recombinant IGF-II was purchased from Bachem, Switzerland and radiolabelled by the chloramine-T method [6]. [¹²⁵I]Insulin was a gift from NOVO Research Institute. Aprotinin and pepstatin were purchased from Sigma, St. Louis, USA. [Methyl 1,2-³H]thymidine was purchased from Amersham, Denmark. Antibody 3637 to rat Man-6-P/IGF-II receptor was a gift from S.P. Nissley, National Cancer Institute, Bethesda, MD, USA.

2.2. Cell culture

Primary neuronal cell cultures were prepared by dissociation of mid-hind brain of 15-day-old rat embryos as described [7].

2.3. Receptor binding

Man-6-P/IGF-II receptors were identified by measurements of binding of [¹²⁵I]IGF-II for 5 h at 4°C in intact and in Triton X-100-solubilized neuronal cells as described [6,7].

2.4. DNA synthesis

Thymidine incorporation in neuronal cells was measured by incubation for 24 h at 37°C with phosphorylated carbohydrates (10 μM–10 mM) or IGF-II (0.1 μM) followed by addition of [³H]thymidine (2 μCi/ml) for 24 h at 37°C [7].

3. RESULTS

3.1. Cellular receptor distribution

The amount of Man-6-P/IGF-II receptors on the cell surface and the total amount of receptors in neuronal cells were determined as the number of IGF-II binding sites in cell monolayers and in Triton X-100-solubilized cells at 4°C. Scatchard analysis of the binding data showed that 28% of the Man-6-P/IGF-II receptors were present on the cell surface (table 1). Of the binding sites, 22% were detergent-insoluble, which may be ex-

Table 1

Man-6-P/IGF-II receptors on intact and solubilized neuronal cells

	Man-6-P/IGF-II receptor number	
	pmol/g cell protein	Percent of total cell receptors
Cell surface receptors	125	28
Total cell receptors	450	100
Detergent-soluble fraction	350	78
Detergent-insoluble fraction	100	22

The values were determined by Scatchard analysis of 125 I-IGF-II receptor binding as described in [6] and are means of two experiments

plained by their association with clathrin-coated pits [8].

3.2. Stimulation of DNA synthesis by Man-6-P

Incubation of neuronal cells with phosphorylated carbohydrates resulted in a twofold increase in the incorporation of 3 H]thymidine compared with mannose (fig.1). Man-6-P and fructose-1-phosphate were equally potent with an ED_{50} of 1 mM, whereas glucose-6-phosphate was 5 times less potent. The effect of Man-6-P was not caused by release of endogenous lysosomal proteases from Man-6-P/IGF-II receptors. Incubation of cells for 2–6 h at 37°C with 5 mM Man-6-P did not increase the proteolytic activity of the culture medium measured by degradation of [125 I]insulin, and addition of protease inhibitors: aprotinin (400 KIE/ml) or pepstatin (1 mM) did not abolish the Man-6-P-induced cell proliferation (data not shown).

3.3. Effect of antibody to Man-6-P/IGF-II receptors

Incubation of cultured neurons with antibody 3637 which inhibits the binding of both IGF-II and β -

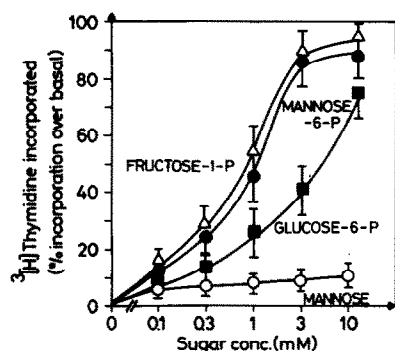


Fig.1. Effect of phosphorylated carbohydrates on DNA synthesis in fetal rat brain neurons. Cells were cultured in the presence of Man-6-P (●), fructose-1-phosphate (Δ), glucose-6-phosphate (■), or mannose (○) at the indicated concentrations for 24 h at 37°C. 3 H]Thymidine (2 μ Ci/ml) was added for additional 24 h of incubation at 37°C and the incorporated 3 H-activity was determined after precipitation with trichloroacetic acid, extensive washing and extraction with 0.2 mol/l NaOH. The data are means \pm SD of 3 experiments.

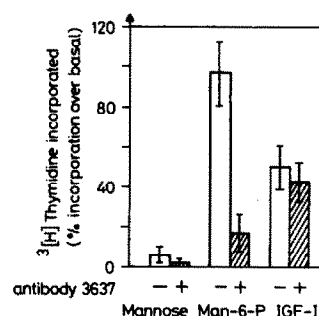


Fig.2. Inhibition of Man-6-P response by antibody to rat Man-6-P/IGF-II receptors. Neuronal cells were preincubated 3 h at 37°C with 1 μ g/ml of control rabbit IgG (open columns) or 1 μ g/ml of anti-Man-6-P/IGF-II receptor IgG 3637 (hatched columns) followed by 24 h culture with 10 mM of mannose, 10 mM Man-6-P, or 0.1 μ M IGF-II. Finally, 3 H]thymidine (2 μ Ci/ml) was added for 24 h and the incorporated radioactivity in DNA measured. Data are means \pm SD of 3 experiments.

galactosidase to Man-6-P/IGF-II receptors [9,10] inhibited the DNA synthesis induced by Man-6-P (fig.2). In control, the effect of IGF-II was not affected confirming our recent findings that the mitogenic response to IGF-II in neuronal cells is mediated by the IGF-I receptor [7]. Finally, the stimulatory effects on DNA synthesis of Man-6-P and IGF-II in submaximally stimulating concentrations were additive, suggesting that different signalling mechanisms are involved (data not shown).

4. DISCUSSION

Our study strongly suggests that Man-6-P-containing proteins act as mitogens in neuronal precursor cells from fetal rat brain by interaction with the Man-6-P/IGF-II receptor. In contrast, IGF-II stimulates neuronal cell proliferation by binding to the IGF-I receptor. These conclusions are based on the following findings. (i) The growth-promoting activity of phosphorylated carbohydrates: Man-6-P = fructose-1-phosphate > glucose-6-phosphate correlates with their potency in inhibiting receptor binding and pinocytosis of β -glucuronidase and phosphorylated oligosaccharides [11,12]. (ii) The stimulation of cell proliferation by Man-6-P is inhibited by antiserum 3637 which is specific for the Man-6-P/IGF-II receptor whereas the effect of IGF-II is unchanged. (iii) The growth-promoting effects of Man-6-P and IGF-II in submaximally stimulating concentrations are additive.

The mitogenic effect of Man-6-P on neuronal precursor cells imply that secreted Man-6-P-containing proteins may act as autocrine or paracrine growth factors during brain development. These proteins include lysosomal hydrolases [2], a major excreted protein of transformed fibroblasts [13], uteroferrin [14], a 52 kDa

protein secreted by mammary cancer cells in response to estrogen [5], and proliferin, a prolactin-related glycoprotein secreted by mouse placenta [15]. Among these proteins, growth activity has only been reported for the estrogen-regulated 52 kDa protein [5]. Attempts to identify phosphomannosylated protein(s) with mitogenic activity on neuronal cells have been initiated in our laboratory, but so far β -galactosidase and proliferin are inactive (F.C. Nielsen, unpublished observation).

In neuronal precursor cells a high proportion (~30%) of the 215 kDa cation-independent Man-6-P/IGF-II receptor is expressed on the cell surface. This number corresponds to the increase of surface receptors in fibroblasts seen after stimulation with insulin, IGF-I, IGF-II, epidermal growth factor and Man-6-P [4], and it may be speculated that increased expression of Man-6-P/IGF-II receptors on the cell surface is associated with signal transduction of the receptor. The Man-6-P/IGF-II receptor has two binding sites for Man-6-P per molecule [16] and a conformational change induced by Man-6-P-containing ligands, may be involved in the cellular response. Little is known about the intracellular signalling mechanism of Man-6-P/IGF-II receptors, but recent studies suggest that the receptor is coupled to effector molecules by G-proteins [1,4].

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