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Mannose-6-phosphate stimulates proliferation of neuronal precursor cells

Finn C. Nielsen and Steen Gammeltoft

Department of Clinical Chemistry, Bispebjerg Hospital, DK-2400 Copenhagen NV, Denmark

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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).

Correspondence address: F.C. Nielsen, Department of Clinical Chemistry, Bispebjerg Hospital, DK-2400 Copenhagen NV, Denmark

2. MATERIALS AND METHODS

2.1. *Chemicals*

Recombinant IGF-II was purchased from Bachem, Switzerland and radiolabelled by the chloramine-T method [6]. [^{125}I]Insulin was a gift from NOVO Research Institute. Aprotinin and pepstatin were purchased from Sigma, St. Louis, USA. [Methyl 1,2-^{3}H]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 [^{125}I]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 [^{3}H]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-

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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-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).

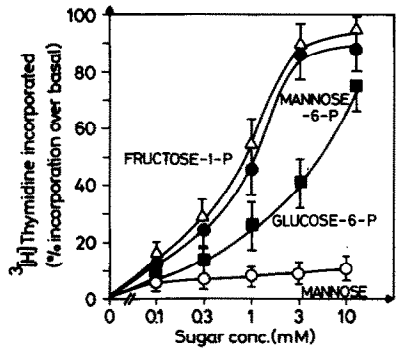

Fig.1. Effect of phosphorylated carbohydrates on DNA synthesis in fetal rat brain neurons. Cells were cultured in the presence of Man-6-P (●), fructose-1-phosphate (△), glucose-6-phosphate (■), or mannose (○) at the indicated concentrations for 24 h at 37°C. [^{3}H]Thymidine (2 μCi/ml) was added for 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 ± SD of 3 experiments.

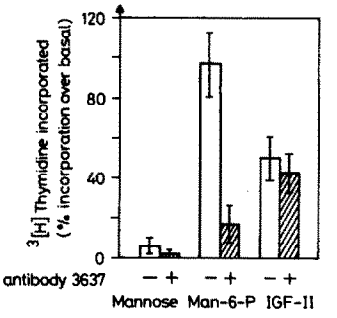

Fig.2. Inhibition of Man-6-P response by antibody to rat Man-6-P/IGF-II receptors. Neuronal cells were preincubated 3 h at 37°C with 1 μg/ml of control rabbit IgG (open columns) or 1 μg/ml of anti-Man-6-PI/IGF-II receptor IgG 3637 (hatched columns) followed by 24 h culture with 10 mM of mannose, 10 mM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

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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