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Coupling of viral membrane proteins to phosphatidylinositide signalling system

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C6 rat glioma cells persistently infected with subacute sclerosing panencephalitis virus (C6/SSPE) were treated with measles antiserum and purified anti-measles IgG. This stimulated phosphoinositide breakdown and an increase in inositol phosphates. In uninfected C6 cells, however, only fetal calf serum (FCS), but not measles antiserum could induce inositol polyphosphate production.

Persistent virus infection; Antimeasles antibody; Phosphinositide turnover; (Glioma cell)

1. INTRODUCTION

Treatment of C6/SSPE cells with measles antiserum has been shown to result in the loss of detectable expression of viral membrane and intracellular proteins [1-5]. The mechanism of this so-called 'antigenic modulation' [1-3,6] is still unknown, but obviously the binding of the antibodies to viral membrane antigens induces a signal that is transmitted into the cells. A possible candidate for such a signal molecule is $PtdInsP_2$, which is located in the cell membrane. $PtdInsP_2$ breakdown has been shown to be involved in quite a number of transmembrane signalling events [7-11]. $PtdInsP_2$ is cleaved by phospholipase C (also called phosphoinositidase [13]) to 1,2-diacylglycerol and $InsP_3$. The first product activates protein kinase C and the second releases Ca^{2+} from intracellular stores. Here we report that purified anti-measles IgG (IgG_m) and monoclonal antibodies against viral HA can induced $PtdInsP_2$ breakdown and an increase in inositol phosphates in C6/SSPE cells, but not in uninfected C6 cells.

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Abbreviations: $PtdInsP_2$, phosphatidylinositol 4,5-bisphosphate; PtdInsP, phosphatidylinositol 4-phosphate; $InsP_3$, inositol 1,4,5-trisphosphate; $InsP_2$, inositol 1,4-bisphosphate; $InsP_1$, inositol 1-monophosphate; HA, haemagglutinin; SSPE, subacute sclerosing panencephalitis; IgG_m, purified anti-measles IgG; FCS, fetal calf serum

2. MATERIALS AND METHODS

Fetal calf serum (FCS) was obtained from Gibco; [^{3}H]inositol from Amersham Buchler (Braunschweig, FRG); phosphoinositide markers were from Sigma (München, FRG); Affi-Gel Protein A from Bio-Rad and Si60 plates from Merck (Darmstadt, FRG).

2.1. *Cell culture*

C6 (ATCC CCL107) and C6/SSPE [16] cells were grown as monolayer cultures in Dulbecco modified Eagle's medium (DMEM) with 5% FCS at 37°C. They were labelled with 5 μCi [^{3}H]inositol for 48 h. To arrest them in G_1 phase they were simultaneously incubated with 0.1% FCS.

2.2. *Analysis of inositol phosphates*

The cells were stimulated by addition of FCS, measles antiserum, monoclonal antibody K83 against measles HR or IgG (IgG_m or bovine IgG), washed with ice-cold PBS and frozen in a dry ice/methanol bath. Subsequently the cells were scraped off the plates with 300 μl methanol and extracted once with $CHCl_3/CH_3OH$/2.4 M HCl (4:3:2). The water-soluble and organic phases were separated by centrifugation.

The water-soluble inositol phosphates were diluted to 10 ml with water and separated by anion-exchange chromatography on 0.5 ml Dowex 1-X8 columns as described by Berridge [18].

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2.3 *Lipid analysis*

After separation of the water-soluble and organic fractions, $CHCl_3$ was removed from the lipids by evaporation under N_2. The phospholipids were separated by thin-layer chromatography on silica gel 60 plates in MeOH/$CHCl_3$/4 M NH_3 (9:7:2) [19,20]. The PtdIns, PtdInsP and PdtInsP$_2$ markers were visualized by iodine vapor, the radiolabelled phosphoinositides by autoradiography, being scraped into vials and scintillation counted.

2.4. *Purification of immunoglobulins*

Blood plasma with measles anti-serum was obtained from patients with subacute sclerosing panencephalitis. Immunoglobulins from human blood plasma were purified by affinity chromatography on Protein A agarose (Affi-Gel Protein A). 1 ml Affi-Gel Protein A was equilibrated with 10 ml binding buffer (10 mM Na_2HPO_4, 0.15 M NaCl, pH 8.2), 1 ml blood plasma from an SSPE patient was applied to the column and this was washed with 10 ml binding buffer. The immunoglobulins were eluted with 0.1 M sodium citrate, pH 3.0, in 1 ml fractions.

3. RESULTS

To serum depleted C6 and C6/SSPE cells were added either fetal calf serum (FCS) or measles antiserum (50 HAI units). As shown in fig.1A the intracellular concentration of $InsP_1$, $InsP_2$ and $InsP_3$ had increased significantly 2 min after addition of FCS both in C6 and C6/SSPE cells. Measles antiserum and monoclonal antibody K83 [12] against measles haemagglutinin induced an even higher increase of inositol phosphates in virus-infected C6/SSPE cells, but had no effect of all on uninfected C6 cells. To exclude that the induction of PtdInsP$_2$ breakdown and $InsP_3$ formation in C6/SSPE cells treated with measles antiserum is due to growth factors present in the blood plasma, we purified IgG by affinity chromatography on Protein A agarose. Purified IgG (50 HAI units) stimulated the production of $InsP_3$ by about 61% within 2 min in C6/SSPE cells, but could not induced any formation of inositol phosphates in C6 cells (fig.1A). This effect is specific for IgGs from measles antiserum, since non-specific immunoglobulins (bovine IgG) did not increase inositolphosphate levels in either C6/SSPE or C6 cells (not shown). The kinetics of inositol phosphate formation in the first 5 min after stimulation by antibodies (measles antiserum or purified anti-measles IgGs) is shown in fig.1B. Maximal levels of $InsP_3$ were reached within 30 s after addition of antibodies, followed by a slow decrease during the next 5 min. $InsP_2$ increased more slowly reaching a

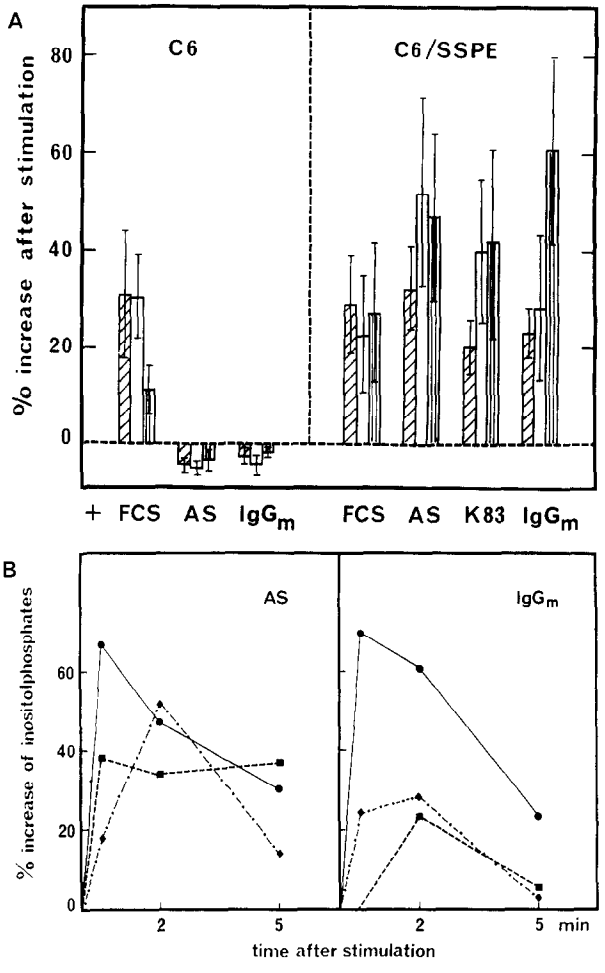

Fig.1. (A) Changes in intracellular concentration of $InsP_1$ (▨), $InsP_2$ (□), and $InsP_3$ (▥) 2 min after stimulation of serum-depleted cells with FCS, measles antiserum (AS), monoclonal antibody K83 or purified anti-measles immunoglobulins (IgG_m). Mean values ± SE of at least 3 determinations. (B) Kinetics of formation of $InsP_1$ (■), $InsP_2$ (♦) and $InsP_3$ (●) after addition of measles antiserum or purified anti-measles IgG. Results are given as % increase compared to unstimulated cells of a representative experiment which was repeated twice.

peak 2 min after stimulation and decreased rather rapidly. Basal levels of inositol phosphates were restored more rapidly after stimulation with IgGs than with plasma containing antiserum.

Fig.2 illustrates the hydrolysis of PtdInsP$_2$ in growth arrested cells after addition of FCS or measles antiserum. In C6/SSPE cells PtdInsP$_2$ was hydrolysed rapidly by addition of FCS as well as by measles antiserum. Already 0.5 min after addition

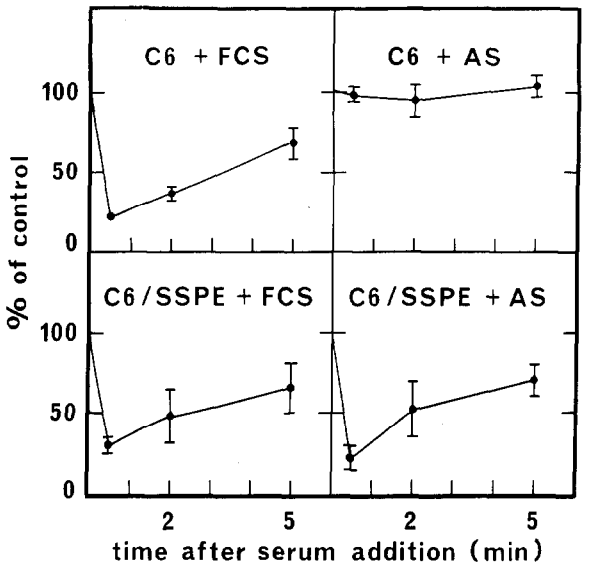

Fig.2. Changes in $PtdInsP_2$ concentration after addition of FCS or measles antiserum (AS) to serum depleted C6 and C6/SSPE cells. Mean values ± SE of 3 determinations.

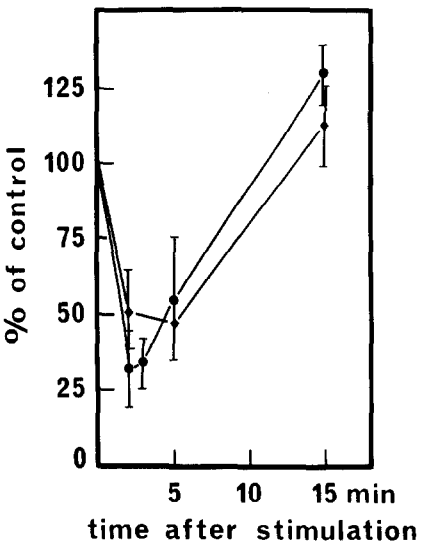

Fig.3. Effects of measles antiserum on $PtdInsP_2$ (●) and PtdInsp (♦) levels in exponentially growing C6/SSPE cells. Mean values ± SE of 3 determinations.

of measles antiserum the $PtdInsP_2$ level had decreased to 21% of the unstimulated control and then slowly increased reaching 71% of control after 5 min (fig.2c, d). Purified IgG could also induce a breakdown of $PtdInsP_2$ to 50% of the control level after 2 min. In uninfected C6 cells, however, $PtdInsP_2$ breakdown could only be stimulated by FCS, not by measles antiserum (fig.2a,b).

Because C6 is a tumor cell line, it is rather difficult to arrest the cells in G_1 phase. This might be a reason for the rather poor stimulation of phosphoinositide turnover after addition of fetal calf serum. Therefore we also tested the effect of measles antiserum on phosphoinositide breakdown in exponentially growing cells (fig.3). Addition of measles antiserum to C6/SSPE cells induced a rapid hydrolysis of PtdInsP and $PtdInsP_2$ to 51% and 32% of the unstimulated control. 10 min after stimulation $PtdInsP_2$ had returned toward control level and had accumulated to 130% after 15 min (fig.3). Purified IgG from measles antiserum could hydrolyse $PtdInsP_2$ in C6/SSPE cells to 54% of the unstimulated control, whereas unspecific immunoglobulins (bovine IgG) had no effect on $PtdInsP_2$ breakdown (not shown).

As shown in fig.4 $InsP_3$ rapidly increased by 76% 1 min after addition of measles antiserum, reaching a maximum of 95% after 3 min, and then gradually decreased to 30% 10 min after stimulation. Addition of purified IgG_m induced a more rapid increase of $InsP_3$ of 104% within 1 min and also a more rapid decrease to control level.

In contrast, measles antiserum or purified IgG_m

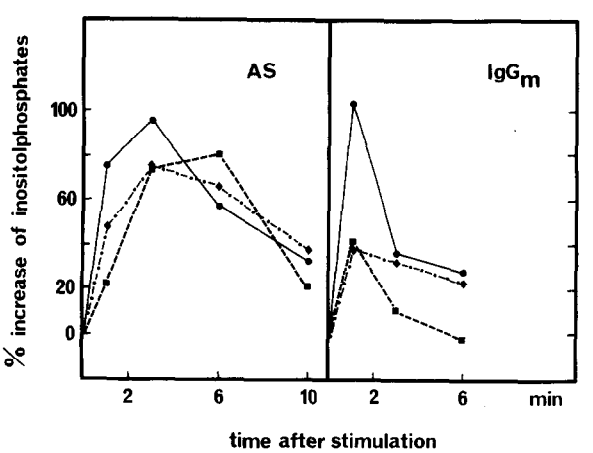

Fig.4. Time course of formation of $InsP_1$ (■), $InsP_2$ (♦) and $InsP_3$ (●) after stimulation of exponentially growing C6/SSPE cells with measles antiserum or purified IgG_m. Stimulation was shown as the % increase compared to untreated control cells. Data are representative of four separate experiments.

could induce neither $PtdInsP_2$ breakdown nor an increase in $InsP_3$ in uninfected C6 cells.

4. DISCUSSION

These data demonstrate that measles antibodies can induce breakdown of $PtdInsP_2$ and increase inositol phosphate formation in cells infected with SSPE virus. This stimulation is specific for measles antibodies and cannot be mimicked by other immunoglobulins, nor can the phosphoinositide turnover be induced by measles antibodies in uninfected cells. These results suggest that viral membrane antigens can behave like specific receptors coupled to the $PtdInsP_2$ hydrolysing signal pathway. The favorite candidate for this receptor-like activity is viral HA, because monoclonal antibody K83 recognizing HA also increases inositol phosphate formation, but we cannot exclude the involvement of the F protein when the stimulation is induced by polyclonal serum. An interesting question is how binding of an antibody to the viral antigen can activate phospholipase C that cleaves $PtdInsP_2$ to $InsP_3$ and diacylglycerol. A regulating guanine nucleotide-binding protein called G_p serves to couple receptors to phospholipase C [14,15]. For several years we have known that measles virus proteins can influence membrane bound G-proteins [1,16]: the activation of the catalytic subunits of adenylate cyclase by the G_s protein is inhibited in C6 cells persistently infected with SSPE virus [4,16,17]. Perhaps there exists a coupling between viral membrane antigens and the G_p protein which can activate phospholipase C after binding of an antibody to the antigen, possibly by conformational changes of the antigen.

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