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INTERFERON-INDUCED ANTIVIRAL STATE IS INHIBITED BY NEOMYCIN AND MIMICKED BY DIACYLGLYCEROLS

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SUMMARY: The antiviral effect of human interferons alpha and beta was inhibited in dose-dependent manner by submillimolar concentrations of neomycin, known to block phosphoinositide hydrolysis and therefore the diacylglycerol formation. On the contrary, the synthetic permeant diacylglycerols (1-oleoyl-2-acetyl-sn or rac-glycerol) were able to induce an interferon-like antiviral state when tested against the vesicular stomatitis virus and herpes simplex type I virus. Hidaka's compound H-8 (1.2 μM), expected to inhibit cAMP- and cGMP-dependent protein kinases, did not modify the antiviral effect of interferon. Our data suggest that the phosphoinositide pathway is involved in transducing the interferon antiviral signal, but, since the exogenous phospholipase C (0.1-1 U/ml) failed to induce an antiviral state, this pathway, although implicated, seems not the only one.

The antiviral action of IFN is well known and extensively studied (see refs. 1-3 for reviews). However, the cellular pathway(s) that translate the IFN-receptor interaction into antiviral state is not established (2,3). It is generally accepted that IFNs alpha and beta share the same receptor and therefore common second messengers are supposed to mediate their actions (3,4). The well known phosphoinositide-derived messengers (IP_3 and DAG; 5, 6) were recently implicated in the antiviral activity of IFN (3,7). Neomycin at

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The abbreviations used are: IFN, interferon; OAG, oleoyl-acetylglycerol; Nm, neomycin; VSV, vesicular stomatitis virus; HSV, herpes simplex type-I virus; FCS, foetal calf serum; H-8, N-(2-(methylamino)ethyl)-5-isoquinoline-sulfonamide; DAG, diacylglycerol; HEF, human embryo fibroblasts; TCID, tissue culture infectious dose; IP_3, inositol-1,4,5-trisphosphate.

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millimolar concentrations was shown to be relatively selective inhibitor of phosphoinositide hydrolysis (8,9), by binding to the substrate (10,11). Thus, Nm became a convenable tool for investigating the contribution of the phosphoinositide pathway in various cellular processes (12-16).

Synthetic diacylglycerols are generally used to mimick DAG-triggered phenomena, via protein kinase C (17). Preliminary results in our laboratories suggested that OAG could be used to induce an antiviral state (18).

Here we report that millimolar concentrations of Nm drastically inhibited the antiviral activity of IFN and that OAG(s) were able to induce an IFN-like antiviral state, the rac-OAG being more potent than the sn-OAG.

MATERIAL AND METHODS

Cells. HEF cells from a continuous cell line were obtained according to established methods (19). Cells were passaged in RPMI-1640 medium supplemented with 10% FCS, glutamine (2 mM), penicillin (100 units/ml). HEF cells were used 5-6 days after seeding when the cultures were confluent.

IFN. Human recombinant IFN alpha-2a was from Hoffman-La Roche Inc. (Nutley, N.J.). Human IFNs alpha and beta induced with Sendai virus on human leucocytes and on human fibroblasts, respectively, were obtained in the Nicolau Institute of Virology according to standard methods (20).

OAG. 1-oleoyl-2-acetyl-rac-glycerol and 1-oleoyl-2-acetyl-sn-glycerol were purchased from Sigma (St. Louis, Mo.).

H-8. Compound H-8: N-(2-(methylamino)ethyl)-5-isoquinoline-sulfonamide was a gift from Prof. H. Hidaka (Mie University, Japan).

Virus replication assay. HEF cells were incubated in the above mentioned medium and pretreated for 1 h with the indicated concentrations of Nm and H-8, respectively. After 1 h the medium was changed and cells were exposed for 18 h to the indicated concentrations of IFN alpha or beta or to OAG as well as to the initial concentrations of Nm and H-8. Cultures were then twice washed and tested for virus reproduction capacity with VSV (Indiana strain) and HSV-I at an input of multiplicity of 1 $TCID_{50}$/cell for both viruses. For infectivity titrations samples of virus taken at the indicated postinfection intervals were serially diluted (tenfold dilutions) in Earle medium and added to plastic multiwell plates (Falcon Plastics, Los Angeles, Calif.) to observe the virus induced cytopathic effect on HEF monolayers.

RPMI-1640 medium, glutamine, FCS were from Flow Labs. (Irvine, Scotland) and phospholipase C (Clostridium perfringens; E.C. 3.1.4.3) and neomycin B sulphate were from Sigma (St. Louis, Mo.).

RESULTS

Fig. 1 shows that Nm inhibited in a dose dependent manner the antiviral effect of IFN alpha. A similar phenomenon was observed when IFN beta was

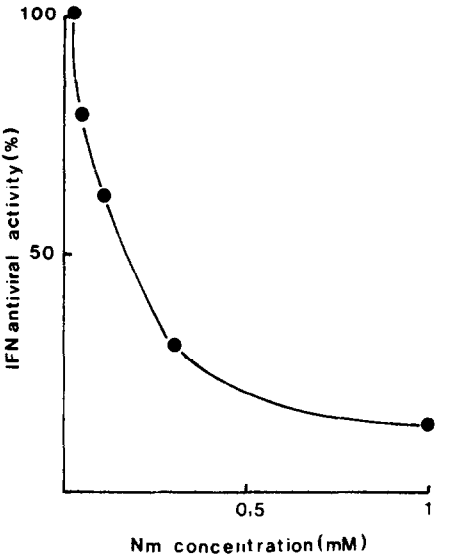

Fig. 1. The inhibitory effect of different concentrations of Nm on the antiviral activity of IFN (A.V.A.) was expressed as: A.V.A. = - lg (VSV titre/VSV titre on IFN pretreated cells). The A.V.A. of 2,000 IU/ml IFN-alpha in the absence of Nm was taken as 100%.

used. Fig. 2 shows that the rac-OAG (both 10 µM and 50 µM) induced an IFN-like antiviral effect against VSV, when the pretreatment time exceeded 10 h. This effect was not obtained when OAG was added simultaneously with VSV or

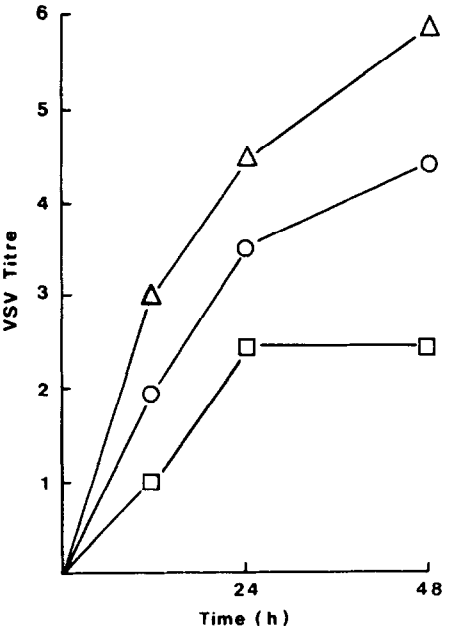

Fig. 2. OAG concentrations of 10 µM and 50 µM (O) induced the same effect on VSV titre (expressed as - lg $TCID_{50}$/0.1 ml) compared with the standard growth curve of VSV (Δ). The shown antiviral effect of 100 IU/ml IFN alpha (□) was not modified by H-8 (1.2 µM).

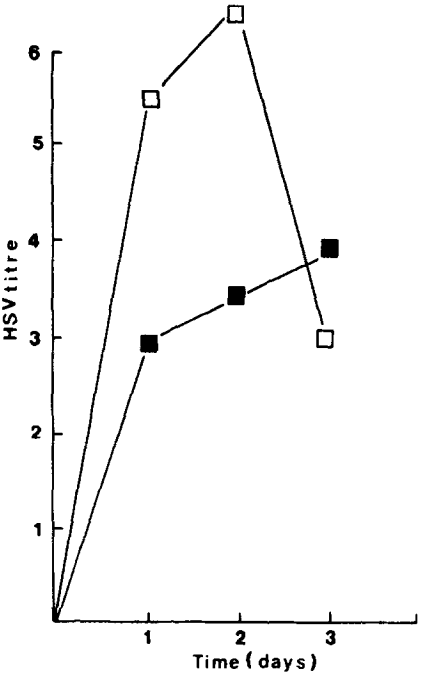

Fig. 3. The antiviral effect of 50 μM OAG (■) when the challange virus was HSV, compared with the standard growth curve of HSV (□). The HSV titre was expressed as - lg $TCID_{50}$/0.1 ml.

30 min before. Fig. 3 shows that rac-OAG had the same antiviral effect when the challenge virus was HSV.

The sn-OAG was also antiviral under the same conditions, but less potent. The antiviral effect of IFNs alpha and beta and of both OAGs remained the same in the presence of 1.2 μM H-8. The addition of exogenous bacterial phospholipase C for 1 h (0.1-1.0 U/ml) failed to induce the expected antiviral effect.

DISCUSSION

Our results show that Nm, used in appropriate concentrations to inhibit polyphosphoinositide hydrolysis and implicitly DAG formation, inhibited the antiviral effect of both IFNs alpha and beta, suggesting that indeed common pathways induce these IFN-dependent antiviral effects. Noteworthy, Nm also produced its inhibitory effect even added 1 h after IFN, showing probably that phosphoinositide turnover is necessary after the IFN-receptor interaction.

Other mechanisms explaining the Nm effect are improbable because Nm does not penetrate into the cell, except for low quantities via endocytosis, which implies many hours or days (21).

The synthetic DAG, OAG was able to induce an interferon-like antiviral state. OAG by itself had no action on VSV or HSV because the simultaneous treatment of HEF cells with OAG and viruses or 30 min pretreatment with OAG had no effect; a latency time was required for inducing the OAG-dependent antiviral state. The action of OAG was indeed IFN-like, since it was reproduced on HSV infection. The rac-OAG was more potent than the sn-OAG, and for both OAGs concentrations over 10 μM did not increase their antiviral effect, thus suggesting that an enzymatic saturable system (protein kinase C ?) is involved. Anyway, the steric configuration of OAG (rac or sn) cannot be further overlooked. Since arachidonic acid is not contained in OAG, the arachidonic acid cascade cannot be implicated in inducing this antiviral state. The antiviral effect of OAG and IFN were not modified by pretreatment with Hidaka's compound H-8 (1.2 μM), known to inhibit cAMP- and cGMP-dependent protein kinases (K_i 1.2 μM and 0.48 μM, respectively; 22). Apparently, our results support current evidence that cAMP and cGMP levels do not correlate with the antiviral effect of IFN (3). Last but not least, the exogenous bacterial) PLC, frequently used to induce phosphoinositide-dependent responses (e.g. 13,23,24), failed to produce any antiviral action, indicating that phosphoinositide hydrolysis is not sufficient for transducing IFN signal.

Finally, since DAG is a common intracellular messenger generated following many agonist-receptor interactions (including IFN) the significance and the mechanism of the antiviral effect of OAG remain to be established.

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