Rabies Virus Infection Selectively Impairs Membrane Receptor Functions in Neuronal Model Cells

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SUMMARY

A persistent infection with rabies virus (HEP-Flury) was established in the CNS-derived hybrid cell line 108 CC15 which possesses specific membrane receptors for prostaglandins, catecholamines and acetylcholine. We report a differential virus influence on the specific receptor response to PGE, isoproterenol and acetycholine as indicated by typical changes of the intracellular cyclic AMP levels.

As the adenylate cyclase activity was unchanged in infected cells in vitro, a selective virus influence on specific receptors themselves or their coupling to the cAMP synthesizing system must be considered.

The mouse neuroblastoma × rat glioma hybrid cell line 108 CC15 (NG-108-15) has been established as a nearly ideal model to study the basic functions of neuronal tissue in vitro (Hamprecht, 1974). Its characteristic properties include a variety of membrane receptors for typical neuro-transmitters such as acetylcholine and catecholamines and hormone-like substances such as prostaglandin E1 (for review, see Hamprecht, 1977). Stimulation of these receptors with their appropriate agonists is followed by well-defined alterations of intracellular cyclic adenosine monophosphate (cAMP) concentrations.

Rabies virus belongs to a group of neurotropic viruses which, after infection of animals, usually persist in the neurons of the central nervous system (CNS) for a long period of time before massive illness and finally death occur. At autopsy, in many cases, nearly all neuron cells in the brain and spinal cord are found to be morphologically intact (Innes & Saunders, 1962; Perl, 1975). Observed histopathological changes in the CNS do not match the drastic dysfunction seen clinically (Murphy, 1977). In clinically manifest rabies infection in man and animals, neutralizing antibodies are often absent at the time of onset of symptoms even though virus infection is then already widespread throughout the CNS. Antibody titres often remain low until the terminal phases of illness. A possible involvement of cell-mediated cytotoxicity or other cytotoxic immune reactions in the pathological events is uncertain (for review, see Murphy, 1977).

In order to study the possible direct action of rabies virus on typical neuronal functions without complicating immunological reactions, we investigated the membrane receptor mediated changes of intracellular cyclic AMP levels in 108 CC15 hybrid cells infected with rabies virus. The cells were cultured at 37 °C in plastic flasks in Dulbecco's modified Eagle's medium (DMEM; GIBCO) containing 10% foetal calf serum, 1 × 10^{-5} m-hypoxanthine (Sigma), 1 × 10^{-5} m-aminopterine (Serva), 1.6 × 10^{-4} m-thymidine (Boehringer; Littlefield, 1964), 0.02 m-glucose and 3 × 10^{-2} m-NaHCO₃ (osmolarity 330 to 340 mOsmol). Cultures were maintained in a water saturated atmosphere of 90% air, 10% CO₂ and cells were passaged by trypsinization (0.005% trypsin). Rabies virus (HEP-Flury strain) was propagated in BHK 21 A cells at 37°C and isolated by the method described by Madore & England (1977) for the ERA strain. Virus infection was performed at a multiplicity of 3 infectious units per cell. After virus adsorption for 45 min at 37 °C in a suspension of 3 × 10⁷
cells/ml DMEM containing 1% FCS and 25 μg/ml DEAE-dextran (Pharmacia, Uppsala), the cells were seeded on to Petri dishes and allowed to grow in fresh medium. After 24, 48, 72 h and after every passage, the percentage of infected cells in culture was estimated by determination of intracytoplasmic ribonucleoprotein (RNP) and membrane-bound virus antigens by direct immunofluorescence technique using FITC-conjugated rabbit antibody against rabies RNP (gift from Dr. Schneider, Tübingen) and FITC-conjugated rabbit anti-rabies serum (Behringwerke, Marburg). Forty-eight hours after infection, 90 to 100% of cultured cells showed virus antigens in their cytoplasm and on their cell membranes and were assayed for their response to receptor stimulation with prostaglandin E₁, isoproterenol and acetylcholine.

Infection of these cells with the rabies virus HEP-Flury strain leads to the development of a productive chronic virus infection with characteristic time-dependent oscillations of the ratio of virus antigen-positive and antigen-negative cells in culture. This has also been reported for rabies virus in other cell cultures and in other rhabdovirus infections (Holland et al. 1976; Palma & Huang, 1974; Kawai et al. 1975). As described for BHK-21 cells, the rabies virus infected 10⁸ CCI 15 cells were not resistant to a virus challenge by heterologous viruses. During the whole observation period of the infected 10⁸ CCI 15 cell population (56 passages over the course of 180 days) no differences in cell morphology, viability or growth rate could be observed as compared to uninfected controls. For tests of the cellular response to membrane receptor stimulation, only cells from an early phase of infection were used in order to avoid possible complications resulting from changes in typical cell properties by multiple passages of the infected cultures. Such changes cannot be excluded completely in hybrid cells in general (Weiss & Ephrussi, 1966) and were in fact demonstrated with the neuroblastoma × glioma hybrid cells (Heumann et al. 1977).

For receptor tests, rabies-infected cells and uninfected controls were freed from growth medium, washed twice and incubated as monolayer cultures with the receptor activating substances, dissolved in serum-free DMEM. After 10 min, the incubation medium was removed and displaced by 5% trichloroacetic acid (TCA) for cell denaturation. The contents of the dishes were carefully collected and centrifuged at 2000 g. The pellet was re-dissolved in 1 M-NaOH and protein was determined according to the method of Lowry et al. (1951). The supernatant was freed from TCA by ether extraction, lyophilized and cyclic AMP determined using the method given by Gilman (1970). Specific cyclic AMP concentrations were calculated as pmol cyclic AMP per mg protein.

Incubation of 10⁸ CCI 15 cells with prostaglandin E₁ (PGE₁) is followed by a rapid rise in intracellular cAMP levels up to a maximum which is reached after about 15 min (Hamprecht & Schultz, 1973; Sharma et al. 1975). Fig. 1 shows that after incubation for 10 min with optimum concentrations of PGE₁, the rise in intracellular cAMP concentrations in rabies virus-infected 10⁸ CCI 15 cells reached only 50% of the levels observed in uninfected controls. Blocking the degrading cyclic nucleotide phosphodiesterase activity by addition of 3-isobutyl-1-methylxanthine (IBMX; Ashcroft et al. 1973; Peytreman et al. 1973; Schultz & Hamprecht, 1973) resulted in higher cAMP concentrations in both virus-infected and uninfected cells but did not alter their relative differences.

Simultaneous incubation of 10⁸ CCI 15 cells with optimal concentrations of PGE₁ and catecholamines led to an inhibition of the cellular cAMP formation. L-Noradrenalin is about 100 times more effective than L-isoproterenol. This is a typical α-adrenergic receptor effect of the adrenergic agonists as shown by Traber et al. (1975b). Fig. 1 shows this α-adrenergic effect in uninfected 10⁸ CCI 15 cells simultaneously incubated with PGE₁ and D,L-isoproterenol and PGE₁ alone. Under the experimental conditions used, the incubation
Fig. 1. Specific intracellular cAMP concentrations in 108 CC15 mouse neuroblastoma × rat glioma hybrid cells incubated with $2.5 \times 10^{-5}$ M-prostaglandin E$_1$ (PGE$_1$) alone, with and without the addition of $1 \times 10^{-5}$ M-3-isobutyl-1-methylxanthine (IBMX) and simultaneously with PGE$_1$ and $2.5 \times 10^{-5}$ M-isoproterenol (Iprot), with and without the addition of IBMX. PGE$_1$ was a gift from the Upjohn Company (Kalamazoo, Michigan, U.S.A.). Shaded columns represent rabies virus (HEP-Flury) infected cells, clear columns refer to uninfected controls. Each column delineates the mean from three independent parallel experiments and shows the standard deviation of the mean (bars).

with D,L-isoproterenol together with PGE$_1$ resulted in a cAMP level nearly 50% lower than with PGE$_1$ alone. This $\alpha$-adrenergic receptor effect was not observed in rabies virus infected 108 CC15 cells. Addition of IBMX resulted in higher cAMP concentrations but did not influence the ratio between levels in infected and uninfected cells.

Fig. 2 demonstrates the typical shifts in intracellular cAMP levels induced by simultaneous incubation with PGE$_1$ and acetylcholine. The latter transmitter binds to the specific muscarinic membrane receptor of the hybrid cells inhibiting the PGE$_1$-induced stimulation of AMP synthesis (Traber et al. 1975a). It is obvious that in rabies virus-infected cells, acetylcholine had quantitatively the same effect on cAMP levels, inhibiting PGE$_1$-induced
Fig. 2. Specific cAMP levels in 108 CC15 cells incubated with 2.5 × 10⁻⁵ M-PGE₁ alone, with and without addition of 1 mM-IBMX and simultaneously with PGE₁ and 1 × 10⁻⁵ M-acetylcholine (ACH), with and without addition of 1 mM-IBMX. Shaded columns represent rabies virus (HEP-Flury) infected cells, clear columns refer to uninfected controls. Each column delineates the mean from three independent parallel experiments and shows the standard deviation of the mean (bars).

rises of this cyclic nucleotide by approx. 50%. Incubation in the presence of IBMX illustrates this situation on a larger scale.

These findings indicate that the normal action of PGE₁ and isoproterenol on the cyclic AMP levels of 108 CC15 cells is impaired following rabies virus infection whereas the cellular response to acetylcholine seems to be unaffected. These effects cannot be caused by different cyclic AMP phosphodiesterase activities under stimulation with different agonists since addition of IBMX, in suitable concentrations, does not alter the relative cAMP levels found under various conditions in the absence of IBMX. The possibility that there could be differences in membrane permeability in uninfected and infected cells for cAMP under the influence of different agonists binding to their specific receptors was excluded (data not shown). Analysis of the fluoride-stimulated adenylate cyclase activity in membrane preparations of rabies virus-infected and uninfected 108 CC15 cells demonstrated that cAMP
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synthesizing enzyme activity was not altered under virus influence (data not shown). A different persistent infected cell system has been described in the previous paper in which an alteration of the specific activity of the adenylate cyclase itself has been observed (Halbach & Koschel, 1979).

From our data on rabies virus-infected 108 CCI5 cells, we have to assume a direct virus influence on specific membrane receptor functions. Whether this virus-induced impairment of normal receptor function is due to a loss in the number of specific receptor molecules, to a decrease in the receptor affinity for the agonists used, or to the obstruction in functional coupling of specific receptors to the adenylate cyclase units, cannot be decided at present. Experiments are in progress to increase our understanding of these mechanisms of virus action.

Many investigators have reported that, especially in cells of the CNS, hormone or transmitter action is followed by characteristic shifts in the intracellular cAMP levels of the target cells. Here, we present evidence that this normal and supposedly important cellular response can be considerably hampered by a persistent non-cytocidal virus infection. The specificity of the viral attack affecting the signal transfer of one agonist but leaving another unchanged provides a new aspect of virus-cell interaction worth considering in the pathogenesis of certain diseases of the nervous system.

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Institut für Virologie und Immunbiologie
der Universität Würzburg
Versbacher Straße 7
D-8700 Würzburg, West Germany

KLAUS KOSCHEL
MICHAEL HALBACH

REFERENCES


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