Distribution of Rabies Virus, Interferon and Interferon-mediated Enzymes in the Brains of Virus-infected Rats

By R. MARCOVISTZ, A. G. HOVANESSIAN and H. TSIANG*
Département de Virologie, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris Cedex 15, France

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SUMMARY

The distribution of rabies virus, interferon and interferon-mediated enzymes, pppA(2'p5'A)n synthetase (2-5A synthetase) and poly(rI)·poly(rC)-Sepharose-bound protein kinase, was studied in different regions of the brains of rabies virus-infected rats. A broad range of virus infectivity was found in the brain stem, cerebellum, cortex, hippocampus and striatum. Similarly, the levels of interferon were variable (2500 to 60000 units/mg protein in tissue extracts) in the different brain regions studied but were unrelated to the corresponding infectivities. The level of 2-5A synthetase and protein kinase was enhanced several-fold in the individual brain regions and the degree of such enhancement was correlated with the level of interferon.

The production of interferon has been detected in the brain of animals during infection by various different viruses (Vilček, 1964; Finter, 1965; Luby et al., 1971; Tongaonkar & Ghosh, 1973). Like these viruses, rabies virus infection of mice results in the induction of interferon in the brain (Stewart & Sulkin, 1966; Wiktor et al., 1972; Marcovitz et al., 1984). This production of interferon is probably responsible for the high levels of interferon circulating in the blood. Interferon in brain extracts and in the plasma of rabies virus-infected mice is of type I: it is stable at pH 2 and is neutralized by antibodies against mouse α + β interferons. In addition to the production of interferon, we have recently shown that the levels of two interferon-mediated enzymes, pppA(2'p5'A)n synthetase (2-5A synthetase) and protein kinase, are enhanced significantly in different organs as well as in the brain of mice infected with rabies virus (Marcovitz et al., 1984). Since similar results (unpublished) have been obtained in rats infected with rabies virus, we now report the regional distribution of virus, interferon, 2-5A synthetase and protein kinase in the brains of virus-infected rats.

The CVS fixed strain of rabies virus, passaged in mouse brain, was used in these experiments. White Wistar rats (70 to 90 g) were inoculated into the footpad and after the onset of paralysis, the rats were killed and the brains were dissected (König & Klippel, 1967; Tsiang, 1982). The infectivity of rabies virus in tissue extracts of the different brain regions was titrated in mice as described in World Health Organization Monograph number 23 (1973). Rat interferon activity was measured by inhibition of the cytopathic effect of vesicular stomatitis virus (VSV) on NRK-49F cells (Wood & Hovanessian, 1979). The activity of interferon in the different regions of the brain was estimated by the level of 2-5A synthetase and protein kinase: both enzymes were assayed after partial purification on poly(rI)·poly(rC)-Sepharose (Hovanessian et al., 1977, 1983).

Table 1 gives the infectivity of rabies virus per mg protein for different regions of the brain assayed in parallel with the level of interferon per mg protein. These were found to be unrelated. The highest level of interferon was detected in the brain stem (60000 units/mg protein) whereas the lowest was found in the striatum (2500 units/mg protein). For the distribution of virus, the brain stem, the cortex and the striatum showed much higher viral infectivity than the cerebellum and the hippocampus. There was little correlation in each of these regions between the level of interferon and the virus infectivity. For example, there were comparable virus titres in the brain stem and cortex but the level of interferon in the cortex was one-third of that in the brain stem.
Table 1. Virus infectivity and levels of interferon, 2-5A synthetase and 67000 M₀ protein phosphorylation in different regions of the brain of rabies virus-infected rats*

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Virus infectivity (LD$_{50}$/mg × 10$^{-2}$)</th>
<th>Interferon (units/mg × 10$^{-3}$)</th>
<th>2-5A Synthetase (nmol/mg/h)</th>
<th>32P-labelled 67000 M₀ protein (c.p.m./mg/assay)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hippocampus</td>
<td>6</td>
<td>7</td>
<td>&lt;0.5</td>
<td>18.5</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>5</td>
<td>42</td>
<td>&lt;0.5</td>
<td>52.6</td>
</tr>
<tr>
<td>Cortex</td>
<td>63</td>
<td>24</td>
<td>&lt;0.5</td>
<td>9.3</td>
</tr>
<tr>
<td>Striatum</td>
<td>28</td>
<td>3</td>
<td>&lt;0.5</td>
<td>5.0</td>
</tr>
<tr>
<td>Brain stem</td>
<td>70</td>
<td>60</td>
<td>&lt;0.5</td>
<td>71.5</td>
</tr>
</tbody>
</table>

* Rats purchased from IFFA-Credo (France) were inoculated with the CVS strain of fixed rabies virus (10⁴ LD$_{50}$ in 0.03 ml aliquots) which was from a 20% brain homogenate from infected mice. Five days after inoculation and coincident with the onset of paralysis, rats were killed and the brain regions dissected with the aid of a stereotaxic atlas. Each dissected brain fragment was washed twice in cold (4 °C) Hanks' solution and stored at -80 °C until the preparation of tissue extracts. Extracts were prepared by homogenization of each brain fragment from three rats in low salt buffer (10 mM HEPES pH 7.6, 10 mM-KCl, 2 mM-magnesium acetate, 100 units/ml aprotinin). Each suspension was then divided into two aliquots; one was centrifuged (1000 g, 15 min) and the supernatant assayed for interferon and rabies virus, whereas the other aliquot was further incubated (5 min, 4 °C) in the presence of 0.5% Nonidet P40 before centrifugation (1000 g, 15 min) and the supernatant assayed for 2-5A synthetase and protein kinase. The different extracts were titrated by mouse inoculation and the results are expressed as 50% mouse intracerebral lethal dose per mg of protein in each extract. The level of interferon was measured on rat cells after treatment at pH 2 (Hovanessian & Rivière, 1980) and the results are expressed in effective units per mg of protein in each extract. The 2-5A synthetase and protein kinase activities were measured after partial purification. The latter was quantified by cutting out the bands of 32P-labelled 67000 M₀ protein from dried gels (using a developed autoradiogram as guide) and counting them in liquid scintillant.

Similarly, the hippocampus and cerebellum showed comparable virus infectivities but the level of interferon was sixfold higher in the latter. The presence of interferon in the brain of rabies virus-infected rats is most probably due to the replication of virus in susceptible neurons. This is in accordance with the results obtained in cell culture: neurons are the predominant cells supporting rabies virus replication when compared with fibroblasts and glial cells (Matsumoto, 1962; Tsiang et al., 1983). Furthermore, rabies virus infection of neuroblastoma cells results in the production of high levels of interferon (unpublished results).

The interferon-mediated protein kinase activity in rat cells, as in mouse cells, is manifested by the phosphorylation of an endogenous 67000 molecular weight (M₀) protein (data not shown). The phosphorylation was enhanced significantly in protein kinase preparations from the brain of rabies virus-infected rats. Similarly, the level of 2-5A synthetase in the brains of infected rats was enhanced by more than 30-fold (18.2 nmol/mg/h) compared to control rats. We used these enzymes as markers (Hovanessian & Rivière, 1980; Krust et al., 1982) to study the immediate response of the different brain regions to interferon. The basal level of protein kinase activity was variable in different regions of normal uninfected rats (Table 1). However, regardless of this basal level there was a several-fold enhancement in individual brain regions in the presence of interferon. This effect became apparent when the ratio of values obtained in enzyme fractions from the different brain regions of infected rats to values observed in the corresponding controls was calculated. Extracts of the individual brain regions from normal rats showed a very low level of 2-5A synthetase. In infected brains, the level of 2-5A synthetase in each region correlated well with the distribution of interferon. As for interferon activity (Table 1), the highest levels of 2-5A synthetase were found in the brain stem while the lowest were in the striatum.

This is the first report on the distribution of interferon and interferon-mediated enzymes in different brain regions following rabies virus infection. Similar experiments were carried out on three different occasions with results comparable to those given in Table 1. The virus infectivity in the different regions of the brain was further monitored by measuring two major viral proteins by an ELISA test (Atanasiu & Perrin, 1979). The results of such analyses indicated that the levels of virus glycoprotein and nucleoprotein are proportional to the titre of virus in the different brain regions (data not shown). Here we have shown that there was a lack of correlation between
the level of interferon and the virus infectivity. It is conceivable, therefore, that neurons differ in
the yield of virus and their production of interferon. It should also be noted that interferon might
diffuse from one section of brain to the other and give misleading results. Thus, the levels of
interferon and virus infectivity should be considered as representative values rather than as
evidence.

Rabies virus infection of rats results in the production of very high levels of interferon in the
brain. This interferon is functional in the induction of 2–5A synthetase and protein kinase in the
different brain regions. Virus replication, however, seems not to be affected since virus
infectivities are high in these animals. Rabies virus-infected rats die in the presence of high
levels of interferon in the circulation and the brain. Kinetic studies of the production of
interferon in rabies virus-infected mice have indicated that the production of interferon in the
brain becomes detectable as soon as the virus starts to replicate there (Marcovitz et al., 1984). It
is possible, therefore, that interferon is produced too late in the infection to exert an efficient
antiviral effect in the brain. In another virus infection, it has been previously shown that
endoogenous interferon induced by lymphocytic choriomeningitis virus might be responsible for
mortality in suckling mice (Rivière et al., 1977). The question of whether the presence of
interferon during rabies virus infection is beneficial or harmful to the host remains to be
answered.

REFERENCES


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