A NOVEL PERMISSIVE ROLE FOR GLUCOCORTICOIDS IN INDUCTION OF FEBRILE AND BEHAVIORAL SIGNS OF EXPERIMENTAL HERPES SIMPLEX VIRUS ENCEPHALITIS

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Abstract—Herpes simplex virus type 1 (HSV-1) encephalitis may present with fever and behavioral changes, to the extent of a psychotic state and psychomotor agitation. We developed a clinically relevant experimental model of HSV-1 encephalitis and investigated host brain responses associated with its clinical signs and whether these responses depend on the presence of circulating glucocorticoids. Intracerebral inoculation of HSV-1 in rats induced fever, motor hyperactivity and aggressive behavior. In adrenalectomized rats HSV-1 failed to induce these signs, although mortality rate was identical to sham-operated rats. Hypophysectomy or blocking glucocorticoid receptors also prevented HSV-1-induced fever. Dexamethasone replacement therapy to adrenalectomized rats restored the HSV-1-induced fever and behavioral abnormalities. HSV-1 inoculation produced hyperproduction of prostaglandin E\(_2\) by brain slices. This effect was abolished in adrenalectomized rats and was restored by dexamethasone treatment. In intact rats HSV-1 induced brain interleukin-\(\beta\) (IL-1\(\beta\)) gene expression. Adrenalectomy alone caused brain IL-1\(\beta\) expression, which did not increase after HSV-1 infection. Similarly, HSV-1-induced brain IL-1\(\beta\) expression in astrocyte cultures. Removal of cortisol from the culture medium caused basal IL-1\(\beta\) mRNA expression which was not increased by infection.

In conclusion, fever, motor hyperactivity and aggressive behavior during experimental HSV-1 encephalitis are dependent on brain responses, including prostaglandin E\(_2\) and IL-1\(\beta\) synthesis. Circulating glucocorticoids play an essential permissive role in the induction of these host brain responses. © 2001 IBRO. Published by Elsevier Science Ltd. All rights reserved.

Key words: adrenocortical axis, viral encephalitis, fever, motor activity, interleukin-1, prostaglandin E\(_2\).

Herpes simplex virus type 1 (HSV-1) is the most common cause of acute, non-epidemic viral encephalitis. The disease presents with fever, behavioral changes, such as psychotic state and focal neurological signs and it still carries significant morbidity and mortality despite antiviral therapy. Several viral genes have been shown to determine HSV-1 virulence for experimental animals. These genes enable the virus to overcome host defense systems (Ben-Hur et al., 1988), to invade the nervous system (Izumi and Stevens, 1990) and to induce lethal encephalitis (Ben-Hur et al., 1987, Chou et al., 1990). However, the mechanisms by which HSV-1 causes the behavioral and clinical signs of encephalitis are still unclear.

We previously showed that brain infection with a neurovirulent HSV-1 strain (but not an avirulent strain) activated the hypothalamic–pituitary–adrenocortical (HPA) axis in rats (Ben-Hur et al., 1995). This activation was dependent on intact noradrenergic input from the brain stem to the hypothalamus (Ben Hur et al., 1996). In addition, the neurovirulent HSV-1 strain induced interleukin-\(\beta\) (IL-1\(\beta\)) and prostaglandin E\(_2\) (PG\(_{\text{E2}}\)) production in the brain (Ben-Hur et al., 1996). These HSV-1-induced brain responses occurred early in the course of infection, while virus titers in the brain were low and in the absence of widespread inflammation or tissue destruction (Ben-Hur et al., 1996). We therefore hypothesized that host brain responses, such as cytokine and prostaglandin production, may play a major role in the development of clinical signs of encephalitis.

Certain brain responses to IL-1, such as PG\(_{\text{E2}}\) synthesis and HPA activation, as well as responses to neurogenic stress do not occur in adrenalectomized animals and are dependent on the presence of systemic glucocorticoids (GC) (Weidenfeld et al., 1989, 1995; Weidenfeld and Feldman, 2000). These and other findings suggested (Munck and Naray-Fejes-Toth, 1994) that basal levels of GC have a permissive role in the induction of immune- and stress-related brain functions. Therefore, in the present study we examined the role of circulating GC in the pathogenesis of HSV-1 encephalitis, using experimental models in which circulating GC were removed either by adrenalectomy or hypophysectomy, or by blocking their receptors.
EXPERIMENTAL PROCEDURES

Animals and viruses

Adult male Sabra rats weighing 180–200 g were housed under artificial illumination (06:00–18:00 h) at an ambient temperature of 22–23°C and supplied with drinking water and food ad libitum. HSV-1 strain Syn17+ was propagated on Vero cells and titrated by plaque assay. 10^3 PFU of HSV-1 (diluted in 20 µl Dulbecco’s modified Eagle’s medium (DMEM)) were inoculated intracerebroventricularly (ICV) into ether-anesthetized rats using a stereotactic device. The coordinates for injection into the lateral ventricle (Pellegrino et al., 1981) were lateral-1.4 mm, anterior-0.0 mm, horizontal-4.2 mm, using the bregma as a reference point. Control animals were injected with uninjected Vero cells suspension. In some experiments the rats were infected 5 days after adrenalectomy or hypophysectomy. After inoculation, the animals were observed daily for clinical signs of disease and body temperature. All animal experiments were approved by the Hebrew University Medical School ethical committee for use and care of animals in research. All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data.

Surgical procedures

Bilateral surgical adrenalectomy was performed under pentobarbital anesthesia (30 mg/kg body weight, i.p.) using the dorsal approach. Rats were provided with 0.5% NaCl in their water source. Surgically hypophysectomized rats were purchased from Harlan laboratories (Israel). Serum adrenocorticotropic hormone (ACTH) and corticosterone (CS) were measured in these rats to ensure completeness of pituitary removal. Only animals in which ACTH and CS serum levels were below 10 pg/ml and 0.5 µg/dl, respectively, were taken for the experiments.

Treatment with steroid compounds

In some experiments adrenalectomized rats were treated daily with dexamethasone i.p. (dexamethasone, Sigma, 200 µg/kg body weight) dissolved in 0.5 ml saline containing 1% ethanol. The treatment began at the day of surgery and continued throughout the experiment period. Control rats were injected with vehicle. The GC receptor type II antagonist RU38486 (donated by Roussel-UCLAF, Romaville, France) was injected subcutaneously daily (25 mg/kg body weight) dissolved in 0.2 ml propylene glycol. The treatment was started 1 day before inoculation with HSV-1 and continued throughout the experiment.

Quantification of the virus in the infected brains

HSV-1 titer in brain tissues was determined by a plaque assay as previously described (Ben-Hur et al., 1983). Following decapitation, the frontal cortex, hippocampus and pons were dissected and frozen until virus determination. Tissues were dounce homogenized in phosphate-buffered saline and serially diluted homogenates were seeded on Vero cells monolayers. The monolayers were covered with 1% agar and after 3 days plaques were counted after Gentian–Violet staining.

Behavioral studies

Our preliminary observations suggested that HSV-1-infected rats exhibit behavioral pathology, including irritability, fearful and aggressive behavior. To quantify these behavioral parameters we examined the responsiveness of the animal to cage opening and insertion of a gloved hand into the cage at 3 days post-infection (p.i.). Behavior was scored on a scale of 0 to 2 as follows: 0 = no response; 1 = overt startle response and attempt to attack the hand; 2 = extreme irritability, fierce attack and attempt to bite the hand and/or jump out of the cage. Because aggressive attack was the most salient symptom we refer to this scale as aggression scale. However, it should be noted that the HSV-1-induced behavioral syndrome also includes irritability and fearfulness. In some experiments motor activity and body temperature were measured continuously using battery-operated biotelemetric transmitters (MiniMitter, OR, USA) that were implanted in the peritoneal cavity during the surgical adrenalectomy or sham operation. Each rat was placed in an individual cage and output was monitored by a receiver board placed under each animal’s cage and fed into a peripheral processor connected to a computer. Two days prior to the experiment, baseline body temperature and activity were measured continuously for 24 h. In some experiments body temperature was measured by a rectal probe connected to a digital thermometer (Type DU-3, Eliab, Copenhagen, Denmark).

Determination of brain IL-1 gene expression

Total RNAs were extracted using the RNeasy kit (Qiagen). After spectrophotometric quantification, 1 µg was reverse-transcribed using an oligo dt primer and the Superscript II enzyme (Life Technologies, Gaithersburg, MD, USA). A 50-µl polymerase chain reaction (PCR) reaction (containing 1.5 mM MgCl₂, 250 ng of each primer, 0.2 mM of dNTP mix and 2.5 units of Taq DNA polymerase (Promega) was performed using 2 µl of the reverse transcription reaction as template. To ensure that these PCR signals detected were not due to amplification of genomic DNA, control reverse transcriptase (RT)-PCR experiments were performed in which cDNA was synthesized without RT. The primers sequences were: β-actin – TGT TAA CCA ACT GGG ACG ATA TGG (+), GAT CTT GAT CAT CTT CAT GGT GCT AGG (–), IL-1β – GTG ATG TTC CCA TTA GAC AGC (+), CTG TCA TCA CAG ACG AGA GG (–). The PCR reaction was run for 35 cycles of 94°C for 45 s, 54°C for 45 s and 72°C for 90 s, followed by a final extension time at 72°C for 7 min. Twenty microliter (for IL-1β) or 5 µl (for β-actin) of the reaction were electrophoresed on a 2% agarose gel and stained with ethidium bromide.

Measurement of brain prostaglandins and serum ACTH and CS

The ex vivo production of PGE₂ was determined in brain slices obtained from the frontal cortex as previously described (Weidenfeld et al., 1995). The tissue slices (10–15 mg each) were placed in 1 ml ice-cold oxygenated Krebs–Ringer buffer (pH 7.4). The supernatants were removed and stored at −80°C until assayed for PGE₂ content. The tissues were homogenized in 1 ml water and protein was determined. PGE₂ was determined by radioimmunoassay as previously described (Weidenfeld et al., 1995). ACTH and CS were measured in peripheral blood by radioimmunoassay as previously described (Weidenfeld et al., 1989).

Astrocyte cultures

Mixed glial cultures were prepared from newborn rat cerebral hemispheres. The hemispheres were dissected, meninges removed and the tissue was trypsinized followed by mechanical dissociation. Debris were removed by centrifugation through a 4% BSA layer. The cells were seeded on poly-L-lysine-coated flasks and cultured in DMEM supplemented with 10% fetal calf serum. After 1 week, a >99% pure astrocytic culture was obtained by shaking the flasks to detach microglia and oligodendrocyte lineage cells, followed by a single passage of the remaining cells. The astrocytic identity of the cells was confirmed by immunocytochemical staining for glial acidic fibrillary protein. In certain experiments the culture medium was changed to a serum free, modified N2 defined medium (Ben-Hur et al., 1998), to which 10 nM Cortisol was either added or omitted. Confluent astrocyte cultures (5 x 10⁵ cells) were inoculated with medium or with HSV-1 at a multiplicity-of-infection of 1–2 plaque-forming units/cell. RNA was isolated from the cells at 6 h p.i. for RT-PCR.
RESULTS

Effect of HSV-1 infection on febrile and behavioral responses in intact and adrenalectomized rats

Sham-operated rats that were injected with \(10^5\) pfu of HSV-1 ICV developed within 24 h clinical signs of encephalitis that included fever (Fig. 1A), increased motor activity (Fig. 2A) and aggressive behavior (Table 1). These signs were maximal in most animals at 48–96 h p.i. Most rats became critically ill between the fourth and sixth day p.i., exhibiting immobility and continuous seizure-like activity and died thereafter. In rats that underwent bilateral adrenalectomy 5 days prior to HSV-1 infection there were undetectable levels of CS (<0.5 \(\mu\)g%) and increased levels of ACTH (340±75 pg/ml versus 15±11 pg/ml in sham-adrenalectomized rats). Adrenalectomy abolished the normal circadian rhythm of body temperature and inhibited the febrile response to HSV-1 infection (Fig. 1B). In addition, HSV-1-infected, adrenalectomized rats did not exhibit motor hyperactivity (Fig. 2B). Ten out of twelve of these rats did not show any sign of fearful and aggressive behavior (Table 1). Although in the HSV-1-infected, adrenalectomized rats the clinical signs of encephalitis, i.e. fever, motor hyperactivity and aggressive behavior were prevented, the mortality rate and its time course were identical to sham-adrenalectomized, HSV-1-infected rats (data not shown).

In order to examine whether adrenalectomized rats may affect viral replication in the brain, HSV-1 titers were determined in various brain regions, including the pons, frontal cortex and hippocampus at 24 h and 72 h p.i. Table 2 shows that HSV-1 titers in the various brain regions of both adrenalectomized rats and sham-operated groups (n = 5 rats/group) did not rise significantly between 24 h and 72 h p.i. In addition, there was no significant difference in virus titers between adrenalectomized rats and sham-operated animals. These results indicate that adrenalectomy did not affect the degree of viral replication in the brain, or the HSV-1-induced mortality. Altogether, our data suggest that the appearance of clinical signs of HSV-1 encephalitis, namely fever and behavioral abnormalities, are dependent on the presence of a functional adrenal gland and are not correlated with viral replication in the brain.

Effect of GC replacement, GC receptor antagonist and hypophysectomy on clinical signs of HSV-1 encephalitis

We next determined whether the inhibitory effect of adrenalectomy on the clinical signs of HSV-1 encephalitis is due to the absence of circulating GC per se, or to other factors associated with adrenalectomy, such as depletion of adrenal medulla catecholamines or hypersecretion of ACTH. To this aim we examined whether GC replacement therapy in adrenalectomized rats can restore the HSV-1-induced fever and behavioral abnormalities. In adrenalectomized rats, which were treated daily with dexamethasone, HSV-1 induced hyperthermia similar to intact rats (Fig. 1B). Similarly, dexamethasone-treated adrenalectomized rats exhibited increased motor activity in response to HSV-1 infection, which was comparable to the hyperactivity of intact HSV-1-infected rats (Fig. 2B). Also, dexamethasone replacement therapy in

<table>
<thead>
<tr>
<th>Table 1. Effects of adrenalectomy and replacement with dexamethasone on HSV-1-induced behavioral pathology</th>
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<tr>
<td>Treatment</td>
</tr>
<tr>
<td>-------------------------------</td>
</tr>
<tr>
<td>Sham</td>
</tr>
<tr>
<td>Adrenalectomized</td>
</tr>
<tr>
<td>Sham</td>
</tr>
<tr>
<td>Adrenalectomized</td>
</tr>
<tr>
<td>Adrenalectomized + dexamethasone</td>
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</table>

Sham-operated and adrenalectomized rats were inoculated with either HSV-1 or vehicle (n = 12/group). Another group of adrenalectomized rats, HSV-1-infected rats received replacement with dexamethasone. The aggression index represents the average aggressive behavior score (see Experimental procedures) for each group. Aggression index score (± S.E.M.) was significantly elevated in sham-HSV and in adrenalectomy-HSV-dexamethasone treated rats, compared to all other groups (\( \chi^2 \) (4) = 12.0, \( P = 0.017 \)).

Table 2. HSV-1 titers\(^a\) in various brain regions of intact (sham-operated) and adrenalectomized rats at 24 and 72 h post ICV inoculation

<table>
<thead>
<tr>
<th></th>
<th>Intact</th>
<th>Adrenalectomized rats</th>
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<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>72 h</td>
</tr>
<tr>
<td>Pons</td>
<td>2.93 ± 0.44</td>
<td>1.66 ± 0.29</td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>2.0 ± 0.0</td>
<td>2.11 ± 0.39</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>4.07 ± 0.67</td>
<td>2.94 ± 0.56</td>
</tr>
</tbody>
</table>

The virus titers in the respective brain regions did not differ significantly between 24 and 72 h p.i. or between intact and adrenalectomized rats.

\(^a\)Data are expressed as log HSV-1 titer/100 mg ± S.E.M.
adrenalectomized rats restored the aggressive behavior induced by HSV-1 (Table 1). Administration of dexamethasone alone did not affect basal body temperature or motor activity of intact, uninfected rats (not shown).

In addition, body temperature was measured in HSV-1-infected rats that were treated with the RU38486 and in rats that underwent hypophysectomy. In intact rats that were treated with RU38486, inoculation with HSV-1 failed to induce fever (Table 3). Similarly, in hypophysectomized rats, which had undetectable levels of both ACTH and CS in the serum, HSV-1 inoculation did not induce fever (Table 3). RU38486 treatment or hypophysectomy alone did not affect significantly the basal body temperature.

Collectively, these results indicate that development of fever and behavioral abnormalities during HSV-1 encephalitis depend on the presence of systemic GCs.

Table 3. Effects of GC receptor blockage with RU38486 and hypophysectomy on HSV-1-induced fever in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Inoculum</th>
<th>Rectal temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>vehicle</td>
<td>37.1 ± 0.2</td>
</tr>
<tr>
<td>Vehicle</td>
<td>HSV-1</td>
<td>38.65 ± 0.57*</td>
</tr>
<tr>
<td>RU38486</td>
<td>vehicle</td>
<td>37.2 ± 0.4</td>
</tr>
<tr>
<td>RU38486</td>
<td>HSV-1</td>
<td>37.65 ± 0.2</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>vehicle</td>
<td>36.57 ± 0.3</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>HSV-1</td>
<td>37.0 ± 0.3</td>
</tr>
</tbody>
</table>

Rectal temperature was measured 72 h post HSV-1 or vehicle administration. Each value represents the mean ± S.D. of five animals per each experimental group. RU38486 or hypophysectomy alone did not significantly change basal rectal temperature. HSV-1 caused a significant increase in temperature in intact but not in RU38486-treated or hypophysectomized rats. *P < 0.05 as compared to all other groups.
Effects of HSV-1 on brain PG\textsubscript{E2} production in intact and adrenalectomized rats and its relation to the febrile response

We have previously shown that HSV-1 infection caused increased production of PG\textsubscript{E2} in various brain regions (Ben-Hur et al., 1996). We therefore examined whether this response depends on the presence of GC. At 3 days p.i. the ex vivo production of PG\textsubscript{E2} was significantly increased (by approximately 2-fold) in tissue slices from the frontal cortex of HSV-1-infected rats as compared to tissues from uninfected control rats (Table 4). Adrenalectomy had no effect on basal production of PG\textsubscript{E2} but it completely prevented the increase in brain PG\textsubscript{E2} synthesis following HSV-1 inoculation. Dexamethasone replacement therapy in adrenalectomized rats fully restored the HSV-1-induced hyperproduction of PG\textsubscript{E2} synthesis in the brain (Table 4).

The lack of a febrile response to HSV-1 in adrenalectomized rats, in which PG\textsubscript{E2} production is impaired, may also be due to inability of the rats to respond to PG\textsubscript{E2}. To examine this possibility adrenalectomized rats or sham-operated rats (n = 4/group) were injected ICV with 1 µg/rat PG\textsubscript{E2} (Sigma, dissolved in 20 µl saline–5% ethanol). Within 1 h both groups showed a comparable increase in rectal temperature, from 37.0 ± 0.2°C to 39.1 ± 0.4°C. In summary, these results suggest that HSV-1-induced fever depends on increased brain production of PG\textsubscript{E2}, which requires the presence of circulating GC. Furthermore, the failure of adrenalectomized rats to develop fever is not due to an impairment in the response to exogenous PG\textsubscript{E2}.

Fig. 2. Effects of adrenalectomy and replacement with dexamethasone on HSV-1-induced motor hyperactivity. For visual clarity, error bars have been left off the curves. S.E.M. in the various groups at different time points was 6.6–13.8% of the mean. (A) In sham-operated rats infected with HSV-1 (HSV) motor activity was significantly higher than in vehicle-treated animals (V), particularly during the dark phase of each day, beginning from the second day p.i. This finding was reflected by a significant group by time interaction (F(38, 114) = 1.66, P < 0.05). (B) In adrenalectomized rats there was no difference in activity between HSV-1 (HSV+V) and vehicle treated rats (V+V). However, dexamethasone (Dex) significantly restored the higher activity levels in HSV-1-infected rats (HSV+dexamethasone), particularly at 42–60 h p.i. This finding was reflected by a significant group by time interaction (F(50, 175) = 2.57, P < 0.01).
We therefore examined whether endogenous GC are important for the induction of IL-1 mRNA in HSV-1-infected brains. Fig. 3A shows that HSV-1 induced IL-1β gene expression in the pons of intact rats, as expected. Adrenalectomy alone caused pronounced expression of IL-1β mRNA. This adrenalectomy-induced activation of the IL-1β gene was not associated with febrile or behavioral changes, as shown above (see Figs. 1 and 2). Importantly, HSV-1 infection did not produce a further increase in IL-1β expression in adrenalectomized rats, as determined by semiquantitative RT-PCR. To determine whether IL-1β expression in adrenalectomized rats was maximal or could be further stimulated by a different immune challenge, we injected 50 μg lipopolysaccharide (LPS) (Difco) ICV into adrenalectomized rats and intact rats. LPS induced IL-1β expression in intact rats’ brain and further enhanced its expression in adrenalectomized rats by 2–3-fold, as determined by semiquantitative RT-PCR (Fig. 3). To test the possibility that the lack of fever in HSV-1-infected, adrenalectomized rats is due to an inappropriate response to IL-1β, we injected ICV 100 ng/rat rhIL-1β (18,000 units/μg, kindly donated by Dr. C. Reynolds, NCI) into adrenalectomized rats (n = 4). Within 1 h, the rectal temperature of the animals increased from 37.2 ± 0.2°C to 38.8 ± 0.3°C. These results suggest that during HSV-1 encephalitis the suppressive effect of adrenalectomy on IL-1 production may underlie the inhibition of the febrile response. In contrast, the febrile response to exogenous IL-1 was not affected by adrenalectomy.

**Role of GC in IL-1 expression in astrocytes in vitro**

The above results clearly demonstrate that the presence of endogenous GC is essential for the clinical signs of viral encephalitis as well as for virus-induced IL-1 gene expression and PGE2 production. To further substantiate this concept we examined in vitro the role of GC in mediating virus-induced IL-1β expression in primary astrocyte cultures. Fig. 3C shows that HSV-1 infection induced IL-1β mRNA expression in astrocytes, grown in medium supplemented with cortisol. When cortisol was removed from the medium, IL-1β was spontaneously expressed in uninfected cultures and there was no further increase in IL-1β mRNA levels in response to viral infection. Thus, GC effects on IL-1β gene regulation in vitro mimic closely their effect in vivo.

### Table 4. Effects of adrenalectomy and replacement with dexamethasone on HSV-1-induced ex vivo production of PGE2 by brain slices from the frontal cortex

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Inoculum</th>
<th>PGE2 (pg/mg protein/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>sham</td>
<td>220 ± 20</td>
</tr>
<tr>
<td>Adrenalectomized</td>
<td>sham</td>
<td>210 ± 25</td>
</tr>
<tr>
<td>Sham</td>
<td>HSV-1</td>
<td>393 ± 30*</td>
</tr>
<tr>
<td>Adrenalectomized</td>
<td>HSV-1</td>
<td>200 ± 14</td>
</tr>
<tr>
<td>Adrenalectomized+</td>
<td>HSV-1</td>
<td>400 ± 29*</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td></td>
<td></td>
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</table>

Animals were killed 72 h after HSV-1 infection. Values represent the mean ± S.E.M. of 10 tissue slices taken from five rats per each experimental group. *P < 0.05 as compared to sham, adrenalectomy, adrenalectomy+HSV groups.
Glucocorticoids affect host responses to HSV-1

In this study we show that removal of endogenous GC by adrenalectomy prevented the most prevalent clinical responses to HSV-1 infection in a rat model, including fever and behavioral changes, i.e. motor hyperactivity and aggression. Surgical adrenalectomy may be associated with various changes in the endocrine milieu, such as decreased sympathetic output and increased levels of pro-opioidmelanocatin (POMC)-derived peptides. The fact that GC replacement therapy completely restored the encephalitic signs in adrenalectomized rats suggests that GC play a major role in mediating these effects of HSV-1. Moreover, inhibition of adrenocortical activity by hypophysectomy or blocking GC receptors by RU38486 also prevented the febrile responses to HSV-1, providing further evidence for the importance of GC in mediating HSV-1-induced host brain responses.

The mechanism by which GC exert a permissive effect on brain responses to HSV-1 infection is not clear. It is well established that the febrile response to viral and bacterial infections depends on increased production of brain PGE2 (Coceani and Akarsu, 1998; Stitt, 1986). We found that in adrenalectomized rats HSV-1 brain infection failed to induce PGE2 production and that GC replacement restored this response. On the other hand, ICV administration of PGE2 in adrenalectomized rats caused hyperthermia, indicating that the febrile response to PGE2 is intact. Thus, the presence of GC is essential for the induction of brain PGE2 production by HSV-1 and the subsequent development of fever. Numerous studies have shown that induction of PGE2 synthesis during infection is mediated by cytokines, including brain-derived IL-1β (Blatteis and Sehic, 1998; Dinarello, 1999; Kluger et al., 1995; Saper and Breder, 1994). As IL-1 gene expression is induced in the brain during HSV-1 encephalitis (Ben-Hur et al., 1996; Ben-Hur et al., 1996), we examined whether HSV-1 can activate this gene in the brain of adrenalectomized rats. We found that removal of endogenous GC triggered IL-1 mRNA expression in the pons. This is in agreement with previous studies which demonstrated increased IL-1 gene expression in the hypothalamus (Goujon et al., 1996) and spleen (Pezeshki et al., 1996) of adrenalectomized rats. Interestingly, this activation was not associated with fever or changes in motor activity. However, HSV-1 infection failed to further increase IL-1 expression in adrenalectomized rats’ brain stem. In contrast, ICV administration of LPS, a potent inducer of IL-1 in the brain, caused further increase in the expression of this gene in the brain stem of adrenalectomized rats, confirming previous observations (Goujon et al., 1996). This finding suggests that the failure of HSV-1 to induce the IL-1β gene following adrenalectomy is probably not due to the fact that this gene is already maximally expressed. We further evaluated the permissive role of GC in activation of IL-1 mRNA expression in purified primary rat astrocyte cultures. In accordance with the observation in vivo, we found that in the presence of cortisol in the culture medium, HSV-1 induced IL-1 expression in the cells. The removal of cortisol from the medium resulted in spontaneous activation of IL-1 expression, but prevented further increase in the expression of this gene following HSV-1 infection. Collectively, these results indicate that the presence of GC is permissive for the induction of IL-1 gene expression by HSV-1 in the brain. ICV administration of IL-1 into adrenalectomized rats induced hyperthermia, indicating that the ability to respond to IL-1 remained intact. We therefore suggest that the failure of HSV-1 to cause fever in adrenalectomized rats may be due to its inability to enhance IL-1 expression and PG_E2 production in the brain.

The molecular mechanism underlying the permissive effect of GC on HSV-1-induced IL-1 expression is not known. Ligand-activated GC receptors mediate their effects via multiple mechanisms (Dumont et al., 1998). They modulate directly gene transcription by interaction with GC response elements on the DNA. A GC-responsive element has specifically been identified in the promoter region of the IL-1 gene (Zhang et al., 1997). Activated GC receptors also interact with cellular transcription factors, such as nuclear factor κB (NFκB) and AP-1 complex that modulate cytokine gene transcription (Scheinman et al., 1995). In view of the above it is possible that GC exert both permissive and inhibitory effects on IL-1 gene expression. In addition, it has been shown that the HSV-1 DNA contains a GC-responsive element (Hardwicke and Schaffer, 1997) that may be involved in GCs-induced latent virus reactivation (Halford et al., 1996, Noisakran et al., 1998). It is therefore possible that GC may play a role not only in mediating brain responses to HSV-1 infection but also may affect the virus directly.

The findings that HSV-1 infection induces motor hyperactivity and aggressive behavior do not coincide with the current view that an increase in brain IL-1 causes sickness behavior, manifested by lethargy, somnolence, and reduced motor activity and social exploration (Dantzer et al., 1998; Yirmiya et al., 1999). Noteworthy, a similar discrepancy may also occur in the clinical course of viral encephalitis in humans, namely high levels of brain IL-1 concurrence with hyperactive and aggressive behavior to the extent of a psychotic and agitated state. It is generally accepted that psychotic behavior with psychomotor agitation are caused by a ‘brain storm’ of hypersecretion of catecholamines (in particular dopamine), originating from brain stem nuclei. As HSV-1 has been shown to infect catecholaminergic nuclei in the brain stem and to increase their turnover (Barnett et al., 1993; McLean et al., 1993; Paivarinta et al., 1991), this may be the mechanism that underlies the hyperactive and aggressive behavior during HSV-1 encephalitis. In addition, IL-1 has been shown to increase catecholamines activity in the brain stem (Kabiersch et al., 1988). It is therefore possible that the HSV-1-induced increase in catecholaminergic activity within the brain is related to IL-1 induction in brain stem nuclei. This may override the behavioral suppressive effects of IL-1, which usually characterize sickness behavior.

In conclusion, we developed an experimental model of
HSV-1 encephalitis manifested by fever, motor hyperactivity and aggressive behavior, which are similar to the clinical presentation of human patients. We showed that these signs are mediated by brain responses to infection, including PGF2 and IL-1 synthesis. While it is well established that GC have inhibitory effects on immune and brain functions, basal levels of GC play an essential permissive role in the induction of these host brain responses. In accordance with the view that GC have a permissive role in mediating brain responses to immune challenges (Munck and Naray-Fejes-Toth, 1994), this study provides the first demonstration that endogenous GC mediate the effect of a viral pathogen in causing the clinical syndrome of encephalitis.

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Glucocorticoids affect host brain responses to HSV-1


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