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Propranolol suppresses reactivation of herpesvirus

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Abstract

Herpes simplex virus type 1 (HSV-1) reactivates from the nervous system and causes recurrent disease in end organs such as the eye and the lips. We found that the β-adrenergic receptor blocker, propranolol, reduces HSV-1 reactivation in an animal model. Mice latent for McKrae strain HSV-1 were injected with propranolol or saline once a day for 3 successive days, and subjected to a brief period of hyperthermia on the second day to induce reactivation. Twenty-four hours after the third injection, swabs of the ocular surface and homogenates of the corneas and trigeminal ganglia were analyzed for the presence of infectious virus and viral DNA. Treatment with propranolol significantly decreased the appearance of infectious virus in the tear film, cornea, and trigeminal ganglia ($P < 0.05$, χ^2-test). The results suggest a possible new pharmacologic approach to suppressing herpesvirus reactivation in the nervous system and thereby preventing recurrent disease.

Keywords: Herpes simplex virus; Virus reactivation; Recurrent herpetic disease; Propranolol; Latency

1. Introduction

A variety of stimuli – including physical and chemical insults to the nerve endings in epithelial tissues; ultraviolet irradiation of epithelial surfaces; irritation of the skin epithelial cells, such as occurs following chemical burns, stress, fever, and immunosuppressive drugs – can trigger reactivation of HSV-1 from the nervous system (Schmidt et al., 1985; Straus et al., 1985; Corey and Spear, 1986; Blondeau et al., 1989; Stanberry, 1989; Shimeld et al., 1990; Whitley, 1990; Spruance et al., 1991). Several lines of

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investigation suggest that sympathomimetic amines may modulate viral reactivation in the nervous system (Laibson and Kibrick, 1966; Kwon et al., 1981; Nesburn et al., 1983). Therefore, we conducted experiments to determine if a β-adrenergic receptor blocker, propranolol, could affect the incidence of viral reactivation following heat stress induction in mice.

2. Materials and methods

2.1. Animals

Female BALB/c strain mice, 5–6 weeks of age were obtained from the Jackson Laboratories, Bar Harbor, ME. Throughout these experiments the animals were handled in accordance with the NIH guidelines on the care and use of animals in research.

2.2. Virus

The McKrae strain of HSV-1 was propagated in Vero cells and the plaque forming units/ml (PFU) were determined using CV-1 cells. At the time of inoculation, virus was diluted to a concentration of 3×10^5 PFU/ml in tissue culture medium and used immediately to infect the animals.

2.3. Experimental design

Mice were infected with the McKrae strain of HSV-1 by topical ocular application. The corneas of the mice were gently scratched in a crosshatched pattern with a 25-gauge needle and each eye received a 25-μl volume of the virus suspension at a concentration of 3×10^5 PFU/ml. Following inoculation, the eyelids were gently held closed for 10 s. For the controls, the corneas were scratched and a 25-μl drop of diluent not containing virus was applied using the same procedure.

Viral infection was documented by visual examination 3, 5, and 7 days after infection. The characteristic clinical signs of photophobia, blinking, and periocular exudate were seen in all infected animals. (Deaths due to encephalitis resulted in a loss of 10% or less of the infected animals.) Uninfected animals did not exhibit these clinical signs. Thirty-five days after primary infection, the animals were randomly divided into groups for use in experiments.

Infected and uninfected animals were given intraperitoneal injections of 0.1 ml of propranolol at a concentration of 1 mg/kg of body weight or saline (controls) on 2 consecutive days. On the following day, one group of infected and one group of uninfected animals were subjected to hyperthermia (immersion in 43°C temperature water for 10 min) (Sawtell and Thompson, 1992), after which the propranolol-treated and saline-treated (control) animals were given one additional intraperitoneal injection. Twenty-four hours later all animals were anesthetized by intraperitoneal injection of a xylazine–ketamine mixture, the ocular surface of each eye was swabbed, and the swabs placed into culture. The animals were sacrificed and tissues removed for analysis. The

corneas and trigeminal ganglia of each animal were processed separately. The experiment was repeated 3 times.

2.4. Ocular swab assay for infectious virus

CV-1 cells were subcultured into 24-well tissue culture plates. The cultures were incubated until confluence was achieved and then the plates were used to assay for infectious virus. Each ocular surface swab was gently swirled in the culture medium in one well of a 24-well plate. The plates were incubated and observed for 21 days and the occurrence of cytopathic effect (CPE) was recorded by an unbiased observer.

2.5. Assay of corneal and trigeminal ganglionic tissue for infectious virus

Tissues were homogenized in 0.2 ml of complete tissue culture medium consisting of RPMI-1640 containing 10% fetal bovine serum supplemented with antibiotics. Following homogenization, the homogenate was diluted to 1 ml, centrifuged at 12,000 *g* for 5 min, and the supernatant plated onto CV-1 indicator cells. All cultures were observed daily for 21 days for the appearance of cytopathic effect (CPE).

2.6. Assay of ganglionic tissue for viral DNA

The pellets obtained from the ganglion homogenates were immediately resuspended in DNA extraction buffer containing proteinase K and detergent and immediately processed for DNA extraction, slot-blotting, and Southern hybridization (Southern, 1975; Thompson et al., 1985; Javier et al., 1987). Pairs of trigeminal ganglia from propranolol-treated and saline-treated control mice were separately homogenized in DNA extraction buffer and the DNA collected for quantitation by spectrophotometry at 260 nm, slot-blotting, and hybridization with a biotinylated probe complementary to the viral DNA polymerase gene. The hybridized probe was detected using a streptavidin alkaline phosphatase conjugate and the adamantyl-disodium 3-(4-methoxyspiro[1,2-dioxetane-3,2-tricyclo[3.3.1.1]decan]-4-yl)phenyl phosphate (CSPD, Tropix, Bedford, MA) substrate.

3. Results

3.1. Effect of propranolol treatment on viral reactivation

In all 3 experiments, fewer of the propranolol-treated animals had infectious virus in the precorneal tear film 24 h after induction of reactivation by hyperthermia, compared with the saline-treated control animals (Table 1). No difference in the time of appearance of CPE was noted in cultures derived from the propranolol-treated and saline-treated controls. Neither infected animals that were not stressed nor uninfected animals had virus in the precorneal tear film. Similarly, fewer corneal and trigeminal ganglionic cultures from propranolol-treated animals contained infectious virus, compared with the

Table 1
Numbers of cultures from the ocular surface, corneal tissue, and trigeminal ganglionic tissue that were positive for herpes simplex virus

Treatment groups	Infectious virus (no. positive/total)		
	Expt. 1	Expt. 2	Expt. 3
Ocular surface			
+ Propranolol	4/14 (29%)	5/11 (45%) [a]	3/12 (25%)
− Propranolol (control)	4/8 (50%)	8/11 (73%) [a]	8/12 (67%)
Cornea			
+ Propranolol	5/14 (36%)	6/11 (55%) [a]	4/12 (33%)
− Propranolol (control)	5/8 (63%)	9/11 (82%) [a]	9/12 (75%)
Trigeminal ganglia			
+ Propranolol	6/14 (43%)	7/12 (64%)	5/12 (42%)
− Propranolol (control)	6/8 (75%)	10/12 (91%)	10/12 (83%)

All of these swabs of the ocular surface and corneal and trigeminal ganglionic tissues were obtained from HSV-1-infected, hyperthermia-stressed mice. χ^2-Analysis of the results in each experiment indicated that there was a statistically significant difference between the numbers of virus-positive cultures from the propranolol-treated animals compared with cultures from the saline-treated control animals ($P < 0.05$). No virus was found in any of the cultures from infected, unstressed mice or from uninfected mice. The denominator represents the number of swabs/tissue samples followed for 21 days.

[a] One culture from this group became infected with bacteria or fungus during the 21-day observation period and was eliminated from the analysis.

tissues from saline-treated control animals (Table 1). Again, infectious virus was not found in the corneal and ganglionic homogenates of infected unstressed animals or uninfected animals.

3.2. Viral DNA in the ganglia of propranolol-treated and control animals

In a comparative analysis of viral DNA in the trigeminal ganglia of propranolol-treated and saline-treated control mice following heat stress-induced reactivation, Southern hybridization of ganglionic DNA extracts revealed a more intense hybridization signal in the extracts of ganglionic DNA from the control mice, compared with the propranolol-treated animals (Fig. 1). Densitometric readings were taken over the areas of the strongest hybridization signals for the DNA from the propranolol-treated and untreated ganglia shown in Fig. 1. The signal density was at least 4 times higher for the DNA from the untreated ganglion, compared with the DNA from the propranolol-treated ganglion. Analysis of ganglionic DNA from two additional experiments gave similar results; there was a two- to four-fold stronger signal in the untreated ganglion DNA sampled, compared with the propranolol-treated ganglion DNA.

4. Discussion

Following infection of end-organ epithelial cells, HSV-1 travels into the peripheral and central nervous system via retrograde axoplasmic flow (Steiner and Kennedy, 1993).

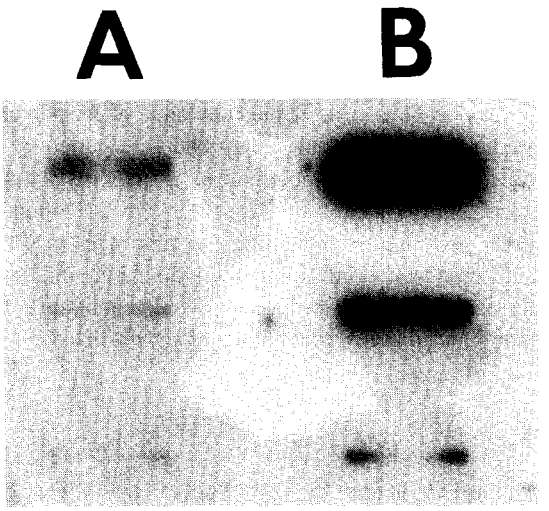

Fig. 1. Slot-blot comparison of viral DNA in the ganglia of propranolol-treated and control mice. Lane A: viral DNA in the ganglia of a propranolol-treated mouse. Lane B: viral DNA in the ganglia of a control mouse.

It appears that neurons in the neural ganglia and in the central nervous system are the permissive hosts for the virus. In such neurons, limited viral replication may occur, followed by the establishment of the latent state (Baichwal and Sugden, 1988; Norgren et al., 1992). This latent state is represented by the presence of viral DNA as an episome (Rock, 1993). Some investigators have speculated that during viral latency there is low-level but continuous replication and production of infectious virus by latent neurons (Latchman, 1990; Mertz, 1990). Although this has yet to be conclusively proven, it could explain the rapid appearance of virus in the end organs following a reactivation stimulus.

Reactivation stimuli include a variety of physical and chemical insults and shocks to the organism as a whole and to the nervous system and nerve endings in particular; however, a common denominator or mediator of viral reactivation has not been identified. The observations of Wilcox and Johnson (1987; 1988) and Wilcox et al. (1990) that nerve growth factor (NGF) plays an important role in the maintenance of viral latency and reactivation generated considerable interest. Ultimately, they showed that 6-hydroxydopamine, a compound that interferes with NGF signal transduction, caused viral reactivation (Wilcox et al., 1990). More recently, cadmium sulfate has been shown to stimulate a high frequency of viral reactivation in mice (Fawl and Roizman, 1993). A common denominator of these investigations appears to be that the viral genome is prevented from functional expression by host factors and that the use of cadmium sulfate, NGF deprivation, and epinephrine circumvent or inactivate the host factors, thereby permitting viral expression.

The results of our study implicate the sympathetic nervous system, specifically adrenergic receptors and their ligands, as modulators of viral latency in the nervous system. β-Adrenergic receptor blockers are used clinically in a number of circum-

stances. In particular, propranolol has been used as a treatment for various cardiac conditions and for migraine. The fact that this compound has been used clinically for a number of years in a large patient cohort suggests that it should be possible to conduct a retrospective chart review to determine if the incidence of herpes labialis is different in the population of patients taking this drug, compared with patients who have not. Such a retrospective analysis, as well as a prospective trial in patients at risk for reactivation of HSV-1, is being planned. In the meantime, laboratory investigations such as the present one may provide direct information regarding the pharmacology of viral reactivation. Much is known about adrenergic stimuli and their blockade and further studies may be able to illuminate the cellular events involved in viral reactivation and lead to a means of blocking these events and thereby preventing recurrent disease.

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