Corneal Sensitivity and Substance P in Experimental Herpes Simplex Keratitis in Mice

Andrew B. Tullo,* Peter Keen,† William A. Blyth,‡ Terry J. Hill,‡ and David L. Easty*

Experimental herpes simplex keratitis in the mouse produced a rapid fall in both corneal sensitivity and levels of corneal substance P (SP). This finding supports the association of SP with sensory neurones and shows that such levels can be used as an indication of damage to neurones resulting, for example, from infection with HSV. However, the delay in recovery of SP compared to the more rapid and complete recovery of sensitivity suggests that SP in the cornea is not directly involved in mediation of the blink reflex. Invest Ophthalmol Vis Sci 24:596-598, 1983

Neuropeptides are currently receiving much attention because of their widespread distribution within the nervous system and because of their possible role in neurotransmission. One of these small peptides, substance P (SP), is known to be particularly strongly associated with primary afferent neurones.1 It is to be found not only at the central terminals of such nerve fibers, but also in the peripheral endings,2 and recently its presence in the cornea was demonstrated by immunohistochemistry3,4 and by radioimmunoassay.5 However, the function of substance P in such peripheral sites is far from clear. By surgical and chemical denervation it has been shown that substance P levels in the cornea are reduced. However, no correlation between changes in SP and corneal sensitivity was found.5 In the human cornea infected with herpes simplex virus (HSV), sensitivity is reduced,6 and this has also been shown to be true of experimental ocular infection.7 In the experiments reported here, SP levels were measured in the cornea of mice with HSV keratitis, first to use SP as an indicator of the integrity of sensory neurones, and second to examine the relationship of corneal SP to corneal sensitivity.

Materials and Methods

Infection of Mice

Eight-week-old outbred male mice, which had been infected with HSV type 1 strain SC16 in the right ear 4 weeks previously,8 were inoculated by corneal scarification of the left eye through 5 μl of growth medium containing 3.5 × 10^6 pfu of the same strain of virus. As controls the corneas of uninfected mice were scarified through medium without virus. Scarification of the cornea in both groups consisted of ten parallel strokes with a 26-gauge needle followed by a further ten perpendicular to the first. Before use all mice were examined with a Zeiss 10SL slit-lamp to exclude any with ocular abnormalities.

Measurement of Corneal Sensitivity

Blink reflex was tested in unanesthetized mice by holding the animal by the scruff of the neck under a dissecting microscope (X10 magnification) and touching the center of the cornea with a sterile filament of nylon (0.24 mm diameter). A 2 cm length of nylon held in a loop holder was found to be convenient to avoid touching whiskers and eyelashes. A scoring system was used where 2.0 = normal brisk reflex, 1.0 = reflex reduced in speed or amplitude, and 0 = absent reflex.

Assay of Substance P

Mice were killed by intraperitoneal injection of pentobarbitone immediately after examination. Both eyes were enucleated, and each was transfixed with a pin for removal of the cornea under a dissecting microscope. Care was taken to remove all conjunctival and limbal tissue. All corneas were treated separately using the right (uninfected) eye as a control. After placing in 200 μl of 10 mM HCl containing 1 mM EDTA, 1 mM dithiothreitol, corneas were boiled and homogenized. The homogenates were freeze-dried, and their SP content was determined by radioimmunoassay using a C-terminal directed antisemur,9 and 125I Tyr8SP as tracer. The test had a sensitivity of 5 pg per tube.

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Results

A group of 45 mice that had been infected as in the Methods section was examined and tested for the blink reflex. At intervals, four to six mice chosen by clinical examination to be representative of the group were killed for SP assay of both corneas (Fig. 1). All mice showed signs of infection varying from transient epithelial lesions to geographic ulceration associated with stromal opacification. Signs were largely reversible, although in the more severe keratitis moderate limbal vascularization and early break-up of the tear film persisted for longer than 14 days.

A marked fall in both corneal sensitivity and SP levels was apparent on the day after corneal inoculation. Minimum sensitivity was reached on day 2, after which it was regained quickly and reached normal values on day 7. The corneal sensitivity of mice assayed for SP corresponded well with that of the remainder of the group (Fig. 1).

Substance P levels fell at the same time as the fall in sensitivity; the minimum occurred on day 4, but the level only reached 70% of normal values by day 11. On day 2 when sensitivity was minimal, three out of six mice assayed for SP had no blink reflex in the infected eye. The mean value of SP in these three corneas was 47% of normal. In a further group of five mice that had shown a complete recovery of corneal sensitivity, corneal SP levels were only 81% of normal values 23 days after infection.

In control mice (four to five daily) whose cornea had been scarified in the absence of virus the level of corneal SP fell to a minimum of 67% of that in the unscarified eye (Fig. 2). On day 7, the last day of observation, the level was 84% of normal. Corneal sensitivity remained normal throughout.

Discussion

The mouse has advantages for studies of ocular herpes simplex in that it is convenient and economical to use relatively large numbers of animals, thus the reactions of a group of animals can be considered together. However, the size of the mouse eye is such that no attempt was made to distinguish between either the center and periphery of the cornea, or between ulcerated and nonulcerated areas when testing for the blink reflex. Primary infection with HSV frequently leads to destructive keratitis and extensive periocular disease. Infection of the immune mouse, however, results in largely reversible disease that is localized to the infected cornea7 and that more closely resembles human herpes simplex keratitis. Both dendritic and geographic ulceration occur associated with a variable degree of stromal infiltration and residual limbal vascularisation. Coincident with signs of acute disease there is a rapid and largely reversible fall in corneal sensitivity.

The most striking finding of the present study was that concurrent with this loss of sensitivity there was a marked fall in levels of corneal SP. In the uninfected cornea, sensitivity was not altered by scarification. Although the minimum SP value was reached on day 4 in both groups of mice, the fall was both more rapid and more pronounced in the infected group. Although slit-lamp examination, aided by Rose-Bengal stain in control mice, revealed no abnormality after 36 hrs, damage to nerve endings as a result of scarification was probably responsible for the fall in corneal SP in these mice. The concomitant fall in corneal sensitivity and the levels of SP after infection with HSV, suggest that the changes underlying both effects are as a result of the disease process. Such a process might involve damage to nerve endings by virus, or by factors released into the tissue during the inflammatory response. Moreover, since virus is transported rapidly to the trigeminal ganglion10,11 it is possible that even by one to two days after infection, damage to the nerve cell body may have contributed to both loss of sensitivity and fall in corneal SP.

The concurrent fall in sensitivity and SP levels supports the association of SP with primary afferent neurons and shows that SP may be used as a marker of neuronal integrity. However, on day 2 SP levels were
Fig. 2. Corneal sensitivity and substance P levels following scarification. Figures are expressed as a percentage of the value of the contralateral (unscarified) eye.

Key words: herpes simplex virus, corneal sensitivity, substance P, keratitis

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References