Capsaicin Pretreatment Prevents Disruption of the Blood-aqueous Barrier in the Rabbit Eye

Gunnel Bynke

Capsaicin, the irritating agent of red pepper, produces ocular inflammation through a neurogenic mechanism. The present study is concerned with the long-term effects of capsaicin pretreatment on the capacity of the eye to respond to different inflammatory stimuli. Following retrobulbar injection of capsaicin to rabbits the aqueous flare response induced by subsequent infrared irradiation (IR) of the iris, subcutaneously administered α-melanocyte-stimulating hormone (α-MSH) and exogenously administered prostaglandin E₂ (PGE₂) was reduced greatly. In the case of IR and α-MSH the reduced responsiveness was manifest for several weeks after capsaicin pretreatment, involving first the capsaicin-treated eye, but later also the contralateral control eye. After 2–3 months the aqueous flare response was normal in both eyes. In the case of PGE₂ the responsiveness was reduced for a shorter time; after 3 weeks the response was normal in both eyes. The results indicate that all three stimuli tested are at least partly dependent upon an intact sensory innervation to disrupt the blood-aqueous barrier, but that the mechanism of action of PGE₂ is different from that of IR and α-MSH. Invest Ophthalmol Vis Sci 24:744-748, 1983

The inflammatory response to trauma of the eye is characterized by initial ocular hypertension, miosis, and breakdown of the blood-aqueous barrier. It is at least partly mediated by antidromic stimulation of sensory nerve fibers, since trigeminal denervation or treatment with the neuronal blocking agent tetrodotoxin prevents or greatly reduces the response. In the rabbit eye, electrical or mechanical stimulation of the trigeminal nerve causes miosis and other signs of inflammation. Prostaglandins (PGs) seem to be involved in the inflammatory response to anterior chamber paracentesis and laser irradiation of the iris, since aspirin treatment prevents inflammation. However, they do not appear to be involved in the response to trigeminal nerve stimulation or in the initial response to nitrogen mustard, both of which require an intact sensory neural pathway to disrupt the blood-aqueous barrier.

Retrobulbar injection of capsaicin, the irritating agent of red pepper, produces ocular inflammation. After normalization, the capsaicin-treated eye is insensitive to the initial hypertensive effect of nitrogen mustard. The desensitizing effect of capsaicin has been attributed to its ability to deplete sensory neurons of substance P (SP). SP has been proposed to act as a mediator of the inflammatory response, since it is thought to be a neurotransmitter in sensory afferents and since exogenous SP is capable of eliciting responses characteristic of inflammation. Furthermore, stimulation of the trigeminal nerve has been shown to release SP into the aqueous humor, whereas trigeminal denervation reduces the SP content of the iris-ciliary body and prevents the inflammatory response.

The present report describes the effect of capsaicin pretreatment on the response to various blood-aqueous barrier disrupting agents: (1) infrared irradiation (IR) of the iris, thought to act partly via prostaglandins, (2) α-melanocyte stimulating hormone (α-MSH), which acts independently of prostaglandins, and (3) PGE₂, which seems to be at least partly dependent upon neural activity to cause inflammation.

Materials and Methods

Adult pigmented rabbits (1.5–3 kg) of mixed strains were used. A 1% capsaicin solution was prepared by dissolving 100 mg of capsaicin (Sigma, St. Louis, MO) in 150 μl of 99.5% ethanol; then 850 μl of Tween 80 was added, followed by 9 ml of saline. Retrobulbar injection of 0.5 ml of 1% capsaicin to the left eye was given as described by Camras and Bito. Control animals were instead given the same volume of the vehicle.

PGE₂ (Leo, Helsingborg, Sweden) was dissolved in...
ethanol (10 mg/ml) and saline was added to give a solution containing 0.5 mg/ml. α-MSH (Ciba, Basel, Switzerland) was dissolved in saline (100 μg/ml). Metohexital sodium (Brietal®, Lilly, Indiana, USA) (10 mg/ml) was used for anesthesia. The animals were given Brietal (5 mg/kg body weight) when injected with capsaicin. No anesthesia was administered during the rest of the experiments.

The blood-aqueous barrier was disrupted by the following methods: 1) IR of the iris for 2 min, 2) subcutaneous injection of α-MSH (20 μg/kg body weight), 3) topical application of PGE2 (2.5 μg) onto the cornea. These doses of α-MSH and PGE2 have previously been found to give an aqueous flare response.14

The course of the barrier damage was followed by measuring photoelectrically the aqueous flare response every 30 min. A correlation between the density of the aqueous flare, expressed in arbitrary units with reference to a standard, and the protein content of the aqueous humor has been demonstrated previously.15

The corneal reflexes were tested by the use of wetted cotton swabs.

Analysis of variance (one-way and two-way) was used for calculating the significance of difference between treated and control eyes and between separate occasions in the same experimental group.

Results

Acute Effects of Capsaicin

In the treated eyes capsaicin produced a prompt inflammatory reaction with conjunctival hyperemia, chemosis, miosis, and a dense aqueous flare, which subsided gradually over a period of 1–2 days (Fig. 1). Even the contralateral eyes responded with a slight aqueous flare. The difference between the eyes was highly significant (P < 0.001).

The pupillary light reflexes and the corneal reflexes were unaffected by capsaicin.

Induction of Aqueous Flare after Capsaicin Pretreatment

When the aqueous flare response to capsaicin had subsided, attempts were made to disrupt the blood-aqueous barrier by IR, α-MSH, and PGE2.

IR: Two to 4 days after pretreatment with capsaicin six rabbits were subjected to IR of both irides (Fig. 2A). The aqueous flare response was greatly reduced in the capsaicin-treated eyes as compared with the contralateral eyes (P < 0.001). Six weeks later three of these rabbits were again subjected to IR of both eyes (Fig. 2B). As compared with the corresponding eyes in Figure 2A the response was now reduced also in the contralateral eyes (P < 0.05) and inhibited to the same degree in the capsaicin-treated eyes (P > 0.05). There was no longer any difference between the capsaicin-treated and the contralateral eyes (P > 0.05).

After 3½ months the procedure was repeated in the same three animals (Fig. 2C). Now the response was no longer inhibited. Thus, as compared with the corresponding eyes in Figure 2A, it had increased in the capsaicin-treated eyes (P < 0.001) and was back to the initial values in the contralateral eyes (P > 0.05). There was no significant difference between the capsaicin-treated and the contralateral eyes (P > 0.05).

α-MSH: Two to 3 days after capsaicin pretreatment of the left eyes α-MSH was injected subcutaneously in five rabbits (Fig. 3A). The aqueous flare response was reduced greatly in the capsaicin-treated eyes as compared with in the contralateral eyes (P < 0.001). Six weeks later three of these animals were again given α-MSH (Fig. 3B). As compared with the corresponding eyes in Figure 3A, the response was now reduced also in the contralateral eyes (P < 0.001) and inhibited to the same degree in the capsaicin-treated eyes (P > 0.05). There was no longer any difference between the capsaicin-treated and the contralateral eyes (P > 0.05). After 2½ months the procedure was repeated in the same three rabbits. Now the aqueous flare response was normal in both eyes (not shown in Fig. 3).

PGE2: Two days after capsaicin pretreatment of the left eyes PGE2 was applied topically to both eyes
of five rabbits (Fig. 4A). The aqueous flare response was reduced greatly in the capsaicin-treated eyes as compared with the contralateral eyes ($P < 0.001$). Three weeks later the same procedure was repeated in all five animals (Fig. 4B). Now the response was no longer inhibited. Thus, as compared with the corresponding eyes in Figure 4A, it had increased in the capsaicin-treated eyes ($P < 0.001$) and was unaltered.

Fig. 2. Aqueous flare response (AFR) to infrared irradiation (IR) of the iris of both eyes 2-4 days (A, $n = 6$), 6 weeks (B, $n = 3$), and 3½ months (C, $n = 3$) after pretreatment with capsaicin of the left eye (dashed curve). Right eye (filled curve) served as control. Ordinate: mean flare density (arbitrary units) ± SE.

Fig. 3. Aqueous flare response (AFR) to subcutaneous injection of α-MSH 2-3 days (A, $n = 5$) and 6 weeks (B, $n = 3$) after pretreatment with capsaicin of the left eye (dashed curve). Right eye (filled curve) served as control. Ordinate: mean flare density (arbitrary units) ± SE.
Fig. 4. Aqueous flare response (AFR) to topically applied PGE$_2$ 2 days (A, n = 5), 3 weeks (B, n = 5), and 2 months (C, n = 5) after pretreatment with capsaicin of the left eye (dashed curve). Right eye (filled curve) served as control. Ordinate: mean flare density (arbitrary units) ± SE.

in the contralateral eyes ($P > 0.05$). There was no longer any difference between the capsaicin-treated and the contralateral eyes ($P > 0.05$). After 2 months the procedure was repeated in the same five animals (Fig. 4C). The aqueous flare response was normal in both eyes. Thus, there was no significant difference between the corresponding eyes in Figure 4C as compared with the corresponding eyes in Figure 4B ($P > 0.05$), and there was no difference between the capsaicin-treated and the contralateral eyes ($P > 0.05$).

At no stage did pretreatment with the vehicle (control animals) interfere with the aqueous flare response to IR, α-MSH and PGE$_2$.

**Discussion**

Retrobulbar injection of capsaicin produced the expected inflammatory response. The fact that the response of the uvea to capsaicin is prevented or greatly reduced by trigeminal denervation or by teatrodotoxin pretreatment suggests that it depends upon an intact trigeminal nerve.

Pretreatment with capsaicin reduced the aqueous flare induced a few days later by IR, α-MSH and PGE$_2$, suggesting that these agents depend upon an intact sensory innervation to disrupt the blood-aqueous barrier. The inhibition of the response to IR and α-MSH persisted for 6 weeks; at this stage the response was reduced also in the contralateral eye. The inhibitory effect of capsaicin was reversible and could not be demonstrated after 2–3 months. Contrary to the effect of IR and α-MSH, the aqueous flare response to PGE$_2$ was normal already 3 weeks after capsaicin. This was surprising in view of the fact that the responses to IR and α-MSH at this stage were greatly reduced. Possibly, the nature of the neural involvement in the response to PGE$_2$ differs from that of the other inflammatory stimuli.

Capsaicin is thought to act on sensory nerve fibers by releasing SP. From this point of view, the lack of response to inflammatory stimuli following capsaicin pretreatment can be explained by degeneration of SP containing trigeminal nerve fibers, which are required for the neurogenic mediation of the inflammatory response. This concept is also consistent with the recent finding that specific SP antagonists inhibit the inflammatory response to IR and bradykinin (Bynke et al, to be published). However, it should be noted that exogenous SP does not completely reproduce the inflammatory response to ocular trauma, in particular with respect to the relative inefficiency of SP in causing aqueous flare. Perhaps SP is not the only neurogenic mediator of inflammation.

**Key words**: experimental ocular inflammation, blood-aqueous barrier, capsaicin, infrared irradiation, α-melanocyte stimulating hormone, prostaglandin E$_2$

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