Sensory Nerve Responses Elicited by Experimental Ocular Hypertension

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In order to clarify the neurophysiological mechanisms underlying the pain sensations that accompany certain forms of glaucoma, the responses of oculary sensory fibers to artificially induced intraocular pressure increases were studied in the cat. In lightly anesthetized animals, intraocular pressure elevations up to 120 mmHg did not evoke the sustained reflex changes in arterial pressure or heart rate that would be suggestive of strong noceptive stimulation. Multiunit activity recorded from filaments of mixed ciliary nerves showed a sharp frequency increase (phasic response) at the onset of intraocular pressure elevations of 20 mmHg or more. In half of the nerves, the discharge stabilized at a higher firing frequency throughout the rise in pressure (tonic response). Corneal units fired phasically in response to intraocular-pressure elevations, and in one third of them this burst of impulses was followed by a low-frequency tonic discharge. Most of the fibers sensitive to light mechanical stimulation of the scleral surface discharged only phasically when intraocular pressure was raised to values of 60 mmHg or more, whereas high threshold scleral fibers were totally insensitive. Iridial fibers responded in all cases to ocular hypertension with a phasic response that became progressively tonic with higher intraocular-pressure values. It is concluded that mechanical deformation of the ocular structures resulting from intraocular-pressure elevations to pathological levels causes only transient excitation of most oculary sensory fibers. Hence, mechanical stimulation appears to be directly responsible only for the transient pain sensations during acute intraocular-pressure increases experienced by glaucoma patients.

Key words: glaucoma; ocular hypertension; ciliary nerves; ocular pain; corneal nerves; scleral nerves; iridial nerves; eye innervation.

1. Introduction

Pain is a sensory modality subserved by nociceptors, i.e. sensory-free nerve endings originating in A-delta or C nerve fibers, that respond to high intensity mechanical, thermal or chemical stimuli (Iggo, 1962; Burgess and Perl, 1967; Perl, 1968; Bessou and Perl, 1969).

One of the possible causes of pain in the eye is glaucoma, a disease characterized by a pathological rise in intraocular pressure (IOP). Ocular hypertension produces a mechanical deformation of eye structures as well as a variable degree of tissue hypoxia and of corneal edema (see Duke-Elder, 1964). It is not known in what measure each one of these potentially damaging stimuli contribute to the pain sensation that appears in certain forms of glaucoma and what types of sensory fibers are excited by the rise in IOP.

Although sensory discharges in the ciliary nerves related to IOP changes have been previously recorded (von Sallmann, Fuortes, Macrì and Grimes, 1958; Lele and Grimes, 1960; Perkins, 1961; Belmonte, Simon and Gallego, 1973), those experiments were directed toward the identification of oculary baroreceptors sensitive to IOP variations within physiological limits (Belmonte et al., 1973) and did not attempt to determine the responsiveness of other classes of sensory receptors to high IOP values.

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In an effort to clarify the underlying neural mechanisms of pain in glaucoma, we have studied the effects of acute IOP elevations on the nervous activity of isolated corneal, scleral and iridal nerve fibers.

2. Materials and Methods

Experiments were performed in 24 adult cats anaesthetized with chloralose-urethane (40 mg kg\(^{-1}\) and 500 mg kg\(^{-1}\), respectively) administered intraperitoneally. Supplementary doses of anaesthetic were injected intravenously through a catheter in the saphenous vein when necessary. Blood pressure, EKG and rectal temperature were continuously monitored, with the temperature maintained at 36–39°C by external heating. Animals breathed spontaneously through a tracheal cannula. Experiments were conducted in conformity with the guiding principles in the care and use of animals approved by the Council of the American Physiological Society.

The head was fixed in a holder, and the superior and lateral walls of the orbital cavity were exposed. The common carotid artery and the maxillary branch of the external carotid artery were dissected; threads were passed around both vessels so that the blood flow in the eye could be reduced by tension on these ligatures. After resection of the extrinsic muscles of the eye, a ciliary nerve was carefully dissected under a binocular microscope and placed on Ag–AgCl electrodes. The nerve was covered with mineral oil and subdissection proceeded until a single identifiable unit could be evoked by natural stimulation. Identification of corneal and scleral fibers was made by mechanical stimulation such as sliding a wet brush over the surface of their receptive fields or touching them with a fine glass rod. The corneal surface was kept moist by saline solution flowing from a reservoir. The exposed scleral surface was protected with moist tissue paper. Impulse discharges were recorded with conventional neurophysiological equipment consisting of an a.c. amplifier with modifiable filters to maximize the signal to noise ratio (usual band pass 100–1500 Hz), oscilloscope and loudspeaker. Data were recorded on FM magnetic tape for off-line analysis. Spikes were also passed through a window discriminator and a D-A converter to be continuously displayed together with IOP (see below) on a paper recorder. In addition, they were fed into a computer to obtain instantaneous frequency and averaged plots of impulses per sec vs. time.

The anterior chamber was cannulated with two 21 G needles. One of them was connected to a pressure transducer for IOP recording, while the other could be connected by means of a stopcock to a 250-ml saline reservoir placed at different heights to artificially vary IOP from 10- to 120 mmHg. In some experiments, a fine glass tube filled with a silver wire that ended in a silver ball was introduced in the anterior chamber; it was used to detect iridial mechanoreceptors by gently displacing the anterior surface of the iris and to stimulate electrically the neuron's receptive field in order to measure its conduction velocity.

The experimental protocol was as follows: Prior to nerve dissection IOP was suddenly elevated from 20- to 40 mmHg for 2–5 min and then returned to 20 mmHg for a 5-min period. The procedure was repeated for 60-, 80-, 100-, 120- and in some cases 140 mmHg. Spontaneously active units or units responding to corneal or scleral mechanical stimulation were then identified, and another cycle of IOP elevations was performed. At the end of IOP elevations of 60 mmHg or more, an arterial occlusion was performed. Individual units located in the cornea, sclera or iris were subsequently identified by further dissection, and the series of IOP elevations and arterial occlusions repeated.

The phasic (or dynamic) part of the impulse response was expressed as the mean frequency per sec during the 5-sec period that followed the IOP elevation. The tonic (static) response was measured as the mean frequency per sec during a time period starting 30 sec after the IOP rise and ending when IOP was returned to control levels.

Conduction velocities of the recorded fibers were calculated from the delay of the evoked response to suprathreshold electrical shocks (0.1 msec, 0.5–3 mA), applied with a pair of silver electrodes to the receptive field and from the conduction distance measured in situ.
3. Results

Effects of IOP elevations on electrocardiogram (EKG) and systemic arterial blood pressures

Elevation of the IOP to values ranging from 40- to 120 mmHg failed to produce consistent changes in arterial pressure or heart rate. To exclude the possibility that reduced sensory input due to deep anesthesia or severance of the recorded nerve were responsible for the absence of reflex cardiovascular changes, the anterior chamber was cannulated in 10 cats under lighter anesthesia (stage III, plane I; see Steffey, 1983), and the arterial pressure and EKG monitored before performing further surgery. A series of IOP elevations (n = 42) extending from 1- to 20 min and at final values 40 to 140 mmHg were carried out, while comparing the mean heart rate and blood pressure with that obtained during the control periods at 20 mmHg. No significant differences were found in heart rate or blood pressure values under these conditions. With IOP elevations over 80 mmHg, a discrete tachycardia accompanied by a slight arterial pressure elevation was observed in four animals; these responses appeared a few seconds after the IOP increase and never lasted more than 1-3 min. Furthermore, these responses could not be systematically repeated. In contrast, other painful stimuli, like forced flexion of a leg, corneal touching or paracentesis of the contralateral eye, evoked a clear blood pressure and cardiac frequency responses. Arterial occlusions were not assayed in these animals.

Effects of IOP elevations on whole nerve activity

Changes in the firing pattern of intact ciliary nerves, secondary to a complete cycle of IOP increases, were explored in 14 nerves obtained from seven different animals. A high-frequency discharge concomitant with the IOP elevation (dynamic or phasic response) were observed in all but two of the explored nerves.

This discharge reached a peak in the first second after the elevation and decayed exponentially, attaining in 15-30 sec a lower discharge level (static or tonic response). In six experiments, the discharge returned to previous control levels within 1-3 min after the onset of the IOP elevation. In another six nerves, the frequency increase persisted as long as the elevated IOP was maintained. Figure 1A is an example of the pattern of frequency change induced by an IOP elevation to 60 mmHg. Notice that a second frequency peak was obtained when IOP was returned to 20 mmHg (off response), suggesting that at least part of the fibers responded to the mechanical deformation of eye structures produced by the pressure change.

The peak frequency of the neural discharge after the IOP elevation, the latency of the frequency increase and the mean frequency per sec attained during the phasic response were roughly proportional to the magnitude of the IOP rise (Fig. 1B). The threshold was 40 mmHg in all experiments, except in two cases in which a response was first elicited at 60- and 80 mmHg, respectively. The mean frequency during the tonic discharge was also related to the magnitude of the IOP increase, reaching frequency levels that could be up to five times those of the control period at IOP values of 80 mmHg or more (Fig. 1B).

Response of corneal fibers to IOP elevations

Responses to IOP elevations were explored in 15 fibers identified as corneal by their response to light mechanical stimulation of the corneal surface. Except for one pure corneal unit, their receptive fields always overlapped onto the fringe of the neighboring
Fig. 1. Frequency changes (impulses per sec) in a whole ciliary nerve in response to intraocular (IOP) elevations. A, Frequency histogram (impulses per sec) obtained in response to an IOP rise from 20- to 60 mmHg (arrows). B, Mean frequency per sec vs. IOP values during a sequence of IOP increases. The filled circles represent the average impulse count during 5 sec following the IOP rise (dynamic response). The triangles represent the mean frequency in impulses per sec during the static period (period extended 30 sec after the IOP rise until the return of IOP to 20 mmHg).

Fig. 2. Nerve impulse discharge (A, upper record) and frequency histogram (B) from a phasic corneal unit in response to an IOP elevation from 20- and 60 mmHg. C, Example of the frequency histogram of a tonic corneal unit after an IOP rise to 40 mmHg. D, Impulse discharge of another tonic corneal unit elicited by IOP elevations from 20 mmHg to 60-, and 80- and 100 mmHg. E, Intensity functions of a tonic corneal unit. Some format as that described for Fig. 1B.
episcleral surface. All of them responded with at least a short burst of spikes when the IOP was suddenly increased, with a threshold of 40 mmHg in eight of the recorded fibers and of 60- and 80 mmHg in the remaining seven units.

In nine corneal fibers, only a phasic response was obtained with IOP increases up to 120 mmHg (Fig. 2A,B). In six additional units, the initial burst of spikes was followed by an irregular discharge that lasted longer and exhibited a higher discharge frequency as IOP was increased (Fig. 2C,D,E). Two of these corneal units exhibited a tonic discharge that persisted for the duration of the stimulus when the IOP was 40 mmHg or greater. An off response consisting of one to five spikes was seen in most fibers when the IOP was suddenly returned to 20 mmHg. In three corneal fibers, at the end of the experiment, saline was directly injected into the anterior chamber (see Methods) to attain IOP values high enough to produce a corneal opacification. However, this maneuver failed to induce an increase in the ongoing tonic discharge after eliciting a brief phasic response.

**Responses of scleral fibers to IOP elevations**

Fibers with a pure scleral receptive field were localized over all the scleral surface. They seemed to be more abundant at the episcleral area and at the posterior pole of the eye surrounding the entrance of the optic nerve, although this point was not thoroughly investigated. Some of the scleral fibers responded to sliding of a wet brush over their receptive fields and were classified as moderate threshold scleral fibers, while others fired only when the scleral surface was indented with a fine glass rod and were called high threshold scleral fibers.

Twenty-five moderate threshold scleral fibers were tested in order to measure their response to IOP elevations. Thirteen were located in the posterior half of the eye, while the remaining had their receptive field in the anterior part of the eye up to the corneoscleral junction. Seventy percent of these superficial scleral fibers had their IOP threshold at 60 mmHg, while the remaining 30% gave a response at 40 mmHg.

The discharge of 21 of these scleral fibers with IOP elevation was composed of a short burst of spikes (Fig. 3A,B) which increased in frequency at higher IOP levels (Fig. 3C). The response usually consisted of a few spikes generated during the first second after the IOP increase followed by a discharge of decreasing frequency that lasted 2–12 sec. In the remaining four fibers, this initial phasic response was followed, after a silent period of several seconds, by an irregular discharge of 0.5–1 impulses per sec, particularly at IOP values over 80 mmHg. Three of these units had their receptive field in the vicinity of the corneoscleral junction and one in the posterior half of the eye. Most moderate threshold scleral fibers responded with an off response composed of a burst of one to five spikes when the IOP was returned to 20 mmHg.

The effects of IOP variations were also tested in six high threshold scleral fibers. All were insensitive to IOP elevations up to 140 mmHg, although they maintain their sensitivity to mechanical stimulation of their receptive fields at the different pressure levels. Conduction velocity was measured in four corneal and four scleral fibers and found to be in the A-delta range (mean value: 12.2 m sec⁻¹).

**Responses of iridial fibers to IOP elevations** (Fig. 4)

Iridial fibers were identified by their response to mechanical stimulation of the anterior surface of the iris with a glass rod introduced in the anterior chamber. The need for keeping an intact anterior chamber precluded the possibility of further characterization of these fibers in terms of their threshold or receptive field dimensions. Mean conduction velocity measured in six of these fibers was 10.5 m s⁻¹.
Fig. 3. Nerve impulse discharge (A, upper record) and frequency histogram (B) of two different scleral units with moderate threshold after IOP increases to 80 mmHg (A, lower record) and 60 mmHg (B), respectively. Arrows in B indicate the onset and the end of IOP elevation. C, Stimulus–response function in another moderate-threshold scleral fiber. Same format as that described in Fig. 1B.

Fig. 4. Response of iridial fibers to IOP elevations. Nerve impulse discharge during successive IOP elevations from 20 mmHg to 40-, 60-, 80- and 100 mmHg. B, Frequency histogram after an IOP elevation to 80 mmHg. C, Stimulus–response function of another unit. Same format as that described in Fig. 1B.

Responses to IOP elevations were obtained in all the explored iridial fibers (n = 16). Thirteen fibers had their firing threshold at 40 mmHg, two at 60 mmHg and one at 80 mmHg. The firing pattern was composed of a short accelerating burst of impulses (phasic response) at threshold. At suprathreshold levels the phasic response was
followed by tonic discharge which increased in duration and frequency with higher pressures. With IOPs of 100 mmHg or more, this discharge persisted along the complete duration of the stimulus.

One of the recorded fibers was identified as iridial because it responded to displacement of the root of the iris; however, it could also be recruited by compression with a glass rod of the corresponding external scleral surface. Its response characteristics to IOP variations were similar to the other iridal fibers. Two additional fibers were detected that fired only when the lens was pushed backwards with the glass rod. One of these units did not respond at all with IOP elevations up to 140 mmHg; the other produced a discharge that increased in its peak frequency and duration with higher IOP values.

Effects of blood flow reductions on nerve activity

In nine scleral fibers, five corneal fibers and 12 iridal fibers, the ipsilateral carotid and maxilar arteries were occluded for 1–2 min following IOP elevations of 60 mmHg or more. No variations of the nervous activity was observed in these units as a consequence of the arterial occlusion, except in one superficial scleral fiber. This unit's receptive field was located in the vicinity of the optic nerve. It responded to either ocular hypertension or blood-flow reduction. The response consisted of a continuous discharge of increasing frequency that stopped abruptly when the blood flow or the IOP were restored to normal (Fig. 5).

![Figure 5](https://via.placeholder.com/150)

**Fig. 5.** Impulse discharge recorded from a moderate threshold scleral fiber in response to decreased ocular blood flow; this fiber started to fire 35 sec after the onset of an IOP elevation to 60 mmHg and stopped shortly after IOP was returned to 20 mmHg (A). When a carotid occlusion was added at the beginning of the IOP increase, the fiber’s discharge did not stop with the IOP reduction, but this occurred as soon as the blood flow was restored (B).

4. Discussion

The functional properties and response characteristics of the various populations of sensory receptors found in the skin of man and cat are quite similar (Hensel and Boman, 1960; Torebjörk and Hallin, 1974; Bessou and Perl, 1969), including the two known groups of high threshold receptors (A-delta and C-fiber nociceptors) whose neural activity seem to encode the occurrence and intensity of stimuli that evoke the sensation of pain (Zotterman, 1939). The same is probably true for other organs,
including the eye; hence, it should be expected that ocular hypertension in cat and human would act on the same types of sensory receptors and elicit in them analogous neural responses.

In our experiments, mechanical stretch of the outer coats of the eye produced by the ocular hypertension causes scleral and corneal sensory fibers to react in different ways. Moderate-threshold scleral units usually responded with a brief phasic discharge, while high-threshold scleral fibers did not fire at all as a consequence of the IOP augmentations. Most of the sensory nerve fibers with a scleral receptive field have been identified as high-threshold A-delta mechano-nociceptors that adapt to mechanical indentation of their receptive field (Gallar, Giraldez and Belonche, 1984). Thus, it is not surprising that maintained stretch of the sclera is not signaled by these fibers and it is doubtful that they are the origin of a sustained pain sensation.

One-fifth of the explored corneal fibers and a few episcleral units responded to the eye distension produced by the pressure change with an irregular, tonic discharge after the initial dynamic burst. This firing pattern is analogous to that obtained in these fibers by suprathereshold mechanical indentation of the corneal surface (Belonche and Giraldez, 1981). The fact that only part of the corneal fibers give a tonic discharge is probably related to the variable degree of mechanical stretch to which they are subjected, according to the anatomical distribution of their receptive endings. Acute corneal edema does not seem to contribute to the excitation of the nerve terminals. The frequent apparition of an off response with the IOP decreases further supports the hypothesis that deformation is the main cause of the corneal nerve excitation during acute IOP elevations. Corneal fibers have been characterized as A-delta nociceptors (Giraldez, Geijo and Belmonte, 1979; Belmonte and Giraldez, 1981) and their stimulation produces in man only irritation and pain (Beuerman and Tanelian, 1979). Hence, excitation of corneoscleral fibers is one of the possible mechanisms to explain pain induced by elevated intraocular pressure.

Stimulation of iridial endings as a consequence of the mechanical distortion of iris and chamber angle during ocular hypertension is another potential source of nociceptive excitation. The root of the iris appears to be richly innervated by nerve fibers that are extremely sensitive to slight mechanical displacement (Saari, Kiviniemi, Johansson and Huhtala, 1973; Gallar et al, 1984). In our experiments, these fibers responded to ocular IOP changes over 60 mmHg with a discharge that may account for a sustained pain sensation at high IOP values.

Carotid occlusion reduces by 35% the blood flow of the eye (Bill, 1974). The summation of carotid occlusion and an IOP elevation to 60 mmHg or more in our experiments should have severely reduced ocular blood flow (Alm and Bill, 1973); further, the resultant tissue ischemia did not seem to alter the ongoing discharge in corneal or iridial nerve fibers. Therefore, ischemia appears not to act as a stimulus for these nerve endings in our experimental conditions. Only in one scleral fiber did ischemia evoke a vigorous discharge. Although this was an isolated observation, the possibility that this mechanism contribute to pain sensations during ocular hypertension cannot be ruled out.

The results indicate that most of the sensory nerve fibers innervating the eye respond phasically to sudden IOP elevations. Only a small fraction of the corneal and iridial fibers seem to maintain a low frequency, tonic discharge in response to very high IOP values. This explains the relatively small increases in total nerve activity observed in the present experiments following IOP augmentations. Strong stimulation of C-fibers has been shown to be correlated with painful sensations in man (Collins,
Nilsen and Randt, 1960) and with pseudoaffective responses which are believed to constitute an expression of pain in animals (Woodworth and Sherrington, 1904). In our recording conditions, only the activity of myelinated fibers was detected and thus the possibility that C-nociceptor activity carrying other type of information has been missed cannot be excluded.

In our experiments in which the anesthetic level was adjusted to eliminate somatic reactivity to pain, maintaining the autonomic responses, no sustained cardiovascular changes could be observed when IOP was raised to 140 mmHg but were elicited by other stimuli that are known vigorously to excite ocular nociceptors (Belmonte and Giraldez, 1981). In the cat, moderate IOP elevations have been shown to cause a reflex activation of autonomic fibers innervating the eye (Gallego and Belmonte, 1974); thus, this autonomic discharge probably participates in the local regulation of ocular blood flow and/or of the aqueous humor dynamics rather than be part of a general autonomic nocifensive reaction. The lack of general autonomic responses to ocular hypertension confirms that the reflex bradycardia induced by external eye compression in animals and man has an extraocular origin (Grandevia, McCloskey and Potter, 1978); it also seems to indicate that ocular hypertension per se is not highly painful in the cat and that in this species the degree of activation of ocular nociceptors evoked by the IOP rises in our experimental conditions is not intense enough to elicit the vegetative responses that appear during other types of pain (Woodworth and Sherrington, 1904; Sato and Schmidt, 1973). This does not need to be the case in humans because the threshold for peripheral nociceptive information to evoke conscious pain sensations can be different. It is well known that the appreciation of pain sensations in man depend on the degree of spatial and temporal summation of the nociceptive input to the central nervous system (Adriaensen, Gybels, Handwerker and Van Hees, 1983, 1984). Nonetheless, the characteristics of acute pain sensations in glaucoma patients are generally congruent with the predominantly phasic pattern of nociceptor discharges observed with ocular hypertension in the cat. Pain is usually absent in open-angle glaucoma, in which in most cases ocular pressure rises very gradually, whereas it often appears during the abrupt IOP rises that occur in angle closure glaucoma and in some forms of secondary glaucoma where the intensity of the sensation is related to the rapidity of the IOP rise (Chandler and Morton Grant, 1979). The sustained pain sensations reported by some of these patients when the IOP remains elevated for periods of time longer than those used in our experiments could be due in part to the processing in the central nervous system of the nociceptive activity originated in corneal and iridial fibers, at a time when a great degree of peripheral adaptation has taken place, as occurs with pain originated in the human skin (Adriaensen et al., 1984). Ocular pain can also be produced by other pathological processes (inflammation, hypoxia) that occur during glaucoma, and that will presumably excite directly the ocular nociceptors contributing perhaps to the maintenance of an ongoing discharge in the corneal and iridial fibers excited mechanically by the ocular hypertension. These are possibilities that need further investigation.

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