Species Differences in the Responses of the Eye to Irritation and Trauma: a Hypothesis of Divergence in Ocular Defense Mechanisms, and the Choice of Experimental Animals for Eye Research

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Information published during the past century, especially the last decade, has identified pronounced species differences, not only in the morphological organization of ocular structures, but also in the functional responses of the eyes of different mammals to experimental and surgical procedures, as well as to drugs and autacoids. For the most part, these differences have been regarded as peculiarities or weaknesses rather than as fundamental evolutionary adaptations optimally suited to the environment and behavior of each species. This paper proposes a working hypothesis of evolutionary divergence in ocular defense mechanisms, based on some of the known morphological and functional differences among mammals, and discusses the implications of these differences with regard to the choice of appropriate animals for use as models in different areas of ophthalmic research.

Key words: eye; mammals; rabbit; primates; aqueous humor; protein; paracentesis; irritation; inflammation; axon reflex; prostaglandins; blood–aqueous barrier; Schlemm's canal; trabecular meshwork; episcleral venous pressure; conjunctival hyperemia; miosis.

Introduction

Much of our understanding of retinal electrophysiology and central visual processes in mammals has been derived from studies on a variety of species, including primates. However, the rabbit has been used primarily, if not exclusively, in most other areas of ocular research, including studies on aqueous humor homeostasis and dynamics, and on the effects of potential ophthalmic drugs. This is particularly surprising in light of the generally accepted view that this species has an atypically labile blood–aqueous barrier (BAB). Indeed, it is virtually impossible to perform experimental procedures on the rabbit eye without inducing a so-called ocular irritative response that includes, in addition to BAB breakdown, pupillary miosis, increased intraocular pressure (IOP), and anterior uveal hyperemia (Duke-Elder and Duke-Elder, 1931; Davson and Huber, 1950; Perkins, 1957; Sears, 1960; Cole and Unger, 1973; Eakins, 1977).

Some investigators have attempted to minimize these effects by shortening their experiments, by reducing the trauma caused by cannulation of the anterior chamber, by pretreating rabbits with drugs presumed to protect against BAB breakdown, or by using intravitreal rather than intracameral drug administration (Sears, 1960; Neufeld, Jampol and Sears, 1972; Bito, Nichols and Baroody, 1982). However, an

Some of the concepts discussed in this paper were presented in my plenary lecture entitled 'Effects of prostaglandins: a second look', delivered to the Vth International Congress of Eye Research in Eindhoven, The Netherlands, 3–8 October 1982. That congress provided my last opportunity to talk with David Cole about subjects of mutual interest. That and many of our previous discussions have played a significant role in the development of some of the concepts and hypotheses presented in this paper. David Cole's death has silenced an important voice in ocular research, but his thoughts and publications will continue to stimulate and guide us.

effective means of preventing an increase in anterior chamber protein levels after paracentesis or cannulation of the anterior chamber of the rabbit eye is yet to be achieved. Since BAB breakdown and other signs of ocular irritation occur to varying degrees after virtually any manipulation of the rabbit eye, the consequent changes in intraocular fluid composition and dynamics greatly affect the interpretation of most experimental findings on the eye of this species.

The acceptance of the rabbit eye as a suitable model for the mammalian eye has apparently been based on the assumption that its sensitivity to irritation constitutes only a quantitative difference in the expression of mechanisms that are identical to those of other species. If this were the case, the extreme sensitivity of the rabbit eye might even offer an experimental advantage over the use of the much less sensitive and much more costly primate eye. However, there is no experimental evidence to support this assumption. This paper examines the possibility that, on the contrary, there are qualitative species differences in the responses of mammalian eyes to trauma – i.e. that evolutionary divergence has led to the development among mammals of different types of eyes that have different morphological and physiological arrangements and respond differently to trauma.

Species Variations in the Responses of the Eye to Irritation

A recent comparative study (Klein and Bito, 1983) showed that shortly after topical administration of nitrogen mustard, a pronounced flare developed in the anterior chamber of the rabbit eye and, to a lesser extent, in that of the guinea pig. This response was somewhat delayed and much less severe in the cat than in the rabbit eye. A similar development of flare was not observed in the eyes of ducks or owl monkeys. These findings are presented schematically in Fig. 1(a).

A marked increase in IOP also followed nitrogen mustard application to the eyes of rabbits, guinea pigs and cats; a smaller increase occurred in the eyes of ducks. However, no increase in IOP was observed within 24 hr after application of the same dose of nitrogen mustard to the eyes of the owl monkeys [Fig. 1(b)]. In rabbits, the IOP increase was biphasic: two episodes of ocular hypertension were separated by a brief period of normal IOP or hypotension (Camras and Bito, 1980a; Klein and Bito, 1983). In guinea pigs, the IOP increase was also biphasic, but the initial increase was smaller and was not followed by a period of hypotension. Indomethacin pretreatment minimized both phases of the IOP rise in guinea pigs and ducks and reduced or blocked the second, but not the first hypertensive phase in rabbits. Indomethacin also reduced or delayed the hypotensive phase that followed the initial pressure rise in cats [Fig. 1(b)], and diminished the development of flare in rabbits and guinea pigs [Fig. 1(a)].

Although topically applied nitrogen mustard did not induce flare and had a striking lack of hypertensive effect on the IOP of owl monkeys, it produced profound pupillary miosis that could not be blocked by indomethacin pretreatment [Fig. 1(c)]. There was also a miotic response in the eyes of rabbits and cats, which was more pronounced and more effectively blocked by indomethacin in the latter. The same doses of nitrogen mustard had no measurable miotic effect on the eyes of ducks or guinea pigs [Fig. 1(c)].

Even more striking species differences have been observed in the responses of vertebrate eyes to ionizing radiation. Exposure of the rabbit eye to as little as 250 rads of X-ray caused severe BAB breakdown (Worgul, Bito and Merriam, 1977) whereas doses four to 40 times greater did not produce a similar effect in the eyes of guinea pigs, cats, monkeys or chickens (Stetz and Bito, 1978; Bito and Klein, 1981).
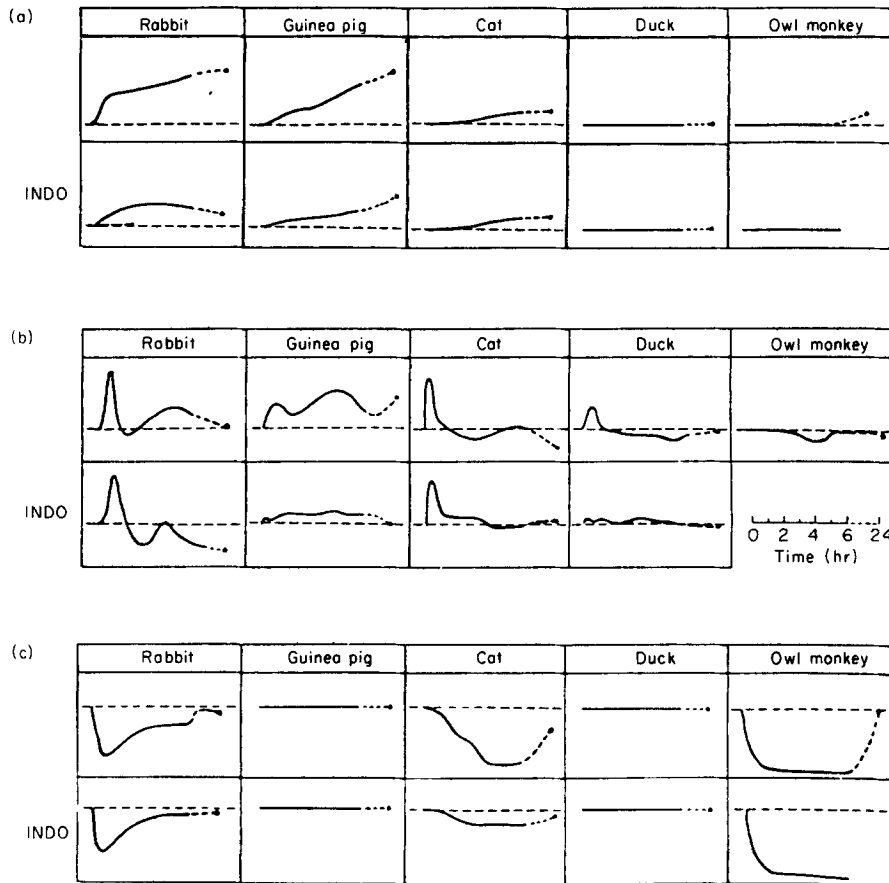
FIG. 1. Schematic representation of the extent and time course of ocular responses within the first 24 hr after topical application of nitrogen mustard solution to the corneal surface of five vertebrates. (a) Flare; (b) intraocular pressure; (c) pupil diameter. In each panel, the second row of graphs refers to ocular responses of animals that were pretreated with a combination of systemic and topically applied indomethacin. (Based on previously published data: Camras and Bito, 1980a, b; Klein and Bito, 1983.)

Dramatic species variations in the reactions of the eye to exogenous prostaglandins have also been reported. The rabbit eye is the most sensitive of the species studied so far and the primate eye the least sensitive, especially to the adverse effects of these autacoids (Bito, 1984).

These observations demonstrate that eyes of different species respond differently to the same irritants. Furthermore, even similar responses are not necessarily mediated by the same mechanism and/or autacoid(s).

Species Variations in the Responses of the Eye to the Loss of Aqueous Humor

Evidence is also accumulating that eyes of different mammals respond differently to the loss of aqueous humor. Anterior chamber paracentesis causes profound BAB breakdown in the rabbit, as demonstrated by the formation of a plasmoid aqueous

and by physiologically and morphologically demonstrable leakiness of the ciliary processes (Kozart, 1968; Unger, Cole and Hammond, 1975). The barrier breakdown is evident primarily in the iridial portions of the ciliary processes of the rabbit (Smelser and Pei, 1965; Kozart, 1968), which are well developed in this species but are virtually nonexistent in primates (Prince, 1964; Kozart, 1968; Ohnishi and Tanaka, 1981).

In contrast, paracentesis-induced protein accumulation in the anterior chamber of rhesus monkeys, and at least some of that in the anterior chamber of cynomolgus monkeys, occurs by reflux through Schlemm's canal (Raviola, 1974; Okisaka, 1976; Bartels, Pederson, Gaasterland and Armaly, 1979), a structure that is highly developed in primates, but which does not exist in the same form in rabbits (Duke-Elder and Wybar, 1961; Tripathi, 1974). Although the existence of such a canalicular reflux mechanism has been questioned by some investigators, its feasibility is supported by numerous observations. For example, Dannheim and Barany (1968) demonstrated reverse flow through the meshwork at a rate of $15\text{--}18\ \mu\mathrm{l\ min}^{-1}$ in some primates when the pressure in a reservoir connected to Schlemm's canal was elevated 5–15 mm Hg above that of the anterior chamber. Furthermore, when IOP falls below the episcleral venous pressure, the lumen of Schlemm's canal is greatly expanded and contains red blood cells (Johnstone and Grant, 1973; Bill, 1977; Moses, 1979).

Although Raviola (1974, 1977) concluded that the ciliary processes are unaffected by paracentesis in rhesus monkeys, others (Okisaka, 1976; Bartels et al., 1979) have reported that, after paracentesis in primates, plasma proteins enter the ocular fluids not only by reflux through Schlemm's canal, but through the ciliary processes as well. However, in contrast to the diffuse morphological changes that occur in the iridial ciliary processes of the rabbit eye after paracentesis, which suggest autacoid-mediated BAB breakdown (Laties, Neufeld, Vegge and Sears, 1976), the morphological changes in the ciliary processes of rhesus eyes after paracentesis have been described as being consistent with mechanical trauma associated with rapid decompression of the globe (Pederson, MacLellan and Gaasterland, 1978). Like reflux of plasma through Schlemm's canal, such decompression-induced breakdown of the ciliary processes could occur in nature only after substantial amounts of aqueous humor had been lost.

The stability of the primate BAB is indeed impressive. For example, Ohnishi and Tanaka (1981) apparently had to perform paracentesis on the eyes of rhesus monkeys four times in order to induce demonstrable damage to the ciliary processes. Even then, the most affected region of the ciliary body showed only selective breakdown of tight junctions, and the total damage appeared to be less than that observed in rabbits after a single paracentesis. The existence of such species differences in ocular defense mechanisms is further supported by the observation that pretreatment with doses of cyclooxygenase inhibitors, which reduced the development of flare in rabbits, failed to do so in rhesus monkeys (Kass, Neufeld and Sears, 1975). Therefore, the highly developed BAB breakdown mechanism of the rabbit eye appears to be nonexistent or vestigial in primates.

On both phylogenetic and morphological grounds, we would expect the human eye to be similar to the eyes of other anthropoid primates with regard to its BAB stability and responses to trauma. This conclusion is supported by several observations. Kronfeld, Lin and Luo (1941) reported that the protein concentration in the human aqueous humor did not increase after sham paracentesis (the bulbar conjunctiva was grasped with forceps and the cornea was punctured) or even after 75 % of the aqueous volume was withdrawn and re-injected within 10 sec. These observations indicate that prophylactic, or even decompression-induced BAB breakdown is not characteristic of

the human eye. On the contrary, these findings are consistent with Raviola's conclusion that, in primates, paracentesis-induced protein entry in the anterior chamber occurs by reflux through Schlemm's canal rather than as a result of BAB breakdown. Clearly, once the BAB has broken down, considerable amounts of protein will enter the anterior chamber, even after normal IOP has been re-established by the re-injection of aqueous humor. In contrast, canalicular reflux will cease immediately after the IOP equals or exceeds the episcleral venous pressure. Based on their experiments with laser irradiation of the iris, Unger, Brown and Edwards (1977) also concluded that the human eye does not exhibit the pronounced and sudden irritative responses that are characteristic of the rabbit eye.

The Apparent Advantages and Disadvantages of a Prophylactic BAB Breakdown Mechanism

Research during recent decades has shown that irritation-induced responses, including BAB breakdown, are mediated in the rabbit eye by complex mechanisms involving neuronal processes and autacoids (Maurice, 1954; Perkins, 1957; Cole, 1961a; Cole and Unger, 1973; Unger, Cole and Bass, 1977; Eakins, 1977; Camras and Bito, 1980a, b). The development of these sophisticated mechanisms cannot be regarded either as accidental or as the result of structural weakness. Therefore, we must consider the possibility that the selective influences of certain habitats and behavioral adaptations have led, in some species, to the development of specific mechanisms for BAB breakdown while, in other species, different habitats and behavioral adaptations have led to the development of a more stable BAB and different mechanisms for protein entry into the anterior chamber. To support this hypothesis, it must be shown that a mechanism that facilitates BAB breakdown is advantageous to some species but disadvantageous to others.

The most pronounced effect of acute BAB breakdown is protein entry into the aqueous humor. One clear advantage of such protein entry is the introduction into the aqueous humor of a clotting mechanism which, in the event of corneal perforation, can minimize the loss of aqueous humor and facilitate re-formation of the anterior chamber. Delivery of plasma proteins into the anterior chamber must be regarded as part of a primary defense mechanism, since neither the cornea nor the aqueous humor has a continuously available clotting mechanism. It can be expected, therefore, that selection for mechanisms that facilitate the prophylactic breakdown of the BAB has occurred in species which, because of their morphological, behavioral, and environmental adaptations, are the most vulnerable to corneal perforation.

Some species, particularly grazing mammals, have evolved a visual system that is well suited for monitoring their environment for predators (Prince, 1956). Such monitoring requires a nearly spherical visual field, which can only be achieved by laterally placed, somewhat protruding, and hence, relatively unprotected globes. Clearly, a deep-seated orbit or a prominent brow ridge would block an animal's view of predators approaching from the side or from overhead.

Rabbits, with their shallow orbits, protruding globes, and high degree of ocular lateralization (Fig. 2) represent an extreme example of the monitoring type of eye (Prince, 1956). The fact that rabbits typically inhabit an underbrush environment makes their eyes especially vulnerable to mechanical trauma. However, the lack of external ocular defenses in rabbits appears to be fully compensated for by a highly developed and exceptionally sensitive intraocular defense mechanism. This conclusion

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