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Effects of sympathetic denervation on the hyaluronan content of the anterior segment in the normal and traumatized rabbit eye

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ABSTRACT.
Purpose: To determine whether there is any involvement of sympathetic nerves in the regulation of ocular hyaluronan production in the normal and traumatized rabbit iris.
Methods: Unilateral sympathetic denervation was performed by removing the right superior cervical ganglion. Hyaluronan concentrations in the iris and aqueous were measured with a radiometric assay at various time intervals after denervation. Peripheral iridectomy was also performed in both denervated and non-denervated eyes.
Results: Hyaluronan concentrations in the iris tissue after denervation were observed to have increased after 1 day, reaching a peak of 129.6 ± 5.7 µg/g wet weight at day 3. Two weeks later, hyaluronan concentrations had fallen back to normal levels. Ocular trauma with peripheral iridectomy in denervated eyes caused an increase of hyaluronan content of up to 253.5 ± 30.5 µg/g wet weight, which was not significantly different from hyaluronan concentrations observed after the same trauma in non-denervated eyes.
Conclusion: Cervical sympathetic denervation results in a moderate increase of the hyaluronan content in the rabbit iris and does not appear to influence the hyaluronan response of the iris to trauma.

Key words: hyaluronan – iris – aqueous humor – sympathetic denervation – trauma response

Hyaluronan (hyaluronic acid, sodium hyaluronate) is a non-sulphated linear polysaccharide. It is richly distributed throughout the body, with the highest concentrations found in connective tissue. High molecular weight exogenous hyaluronan is widely used in ophthalmic anterior segment surgery because of its protective effects on ocular tissues. We are, therefore, interested in examining the biology of endogenous hyaluronan in some detail, and we here report the influence of sympathetic nerves on trauma response.

Endogenous hyaluronan has been identified throughout most of the eye tissues, including the vitreous (Österlin & Jacobson 1968; Laurent 1982), the retina (Egli & Graber 1996), the ciliary body, sclera, conjunctiva and ciliary zonules (Lütjen-Drecoll et al. 1990), the corneal endothelium and the iris (Lütjen-Drecoll et al. 1990; Målander et al. 1993; Koralewska-Makár et al. 1998).

The physiological role of endogenous hyaluronan appears to be associated with trauma and inflammation. In our previous studies, we were able to show a rapid increase of hyaluronan concentration in the iris after peripheral iridectomy and after laser irradiation (Koralewska-Makár et al. 1998, 2001). The changes in aqueous hyaluronan concentration corresponded to similar changes in the iris. In another study, we were able to demonstrate in situ production of hyaluronan by normal and wounded iris tissue in vivo as well as in culture (Koralewska-Makár et al. 2000). The iris appears to be the most important source of aqueous hyaluronan.

The sympathetic innervation present in the anterior segment of the eye (Ehinger et al. 1969; Ruskell 1982) influences a number of important processes. Sympathetic denervation of the eye decreases proliferation of normal and wounded corneal epithelium in rats (Jones & Marfurt 1996), enhances the response of the eye to ocular injury (neutral formaldehyde: Rootila et al. 1987; laser burn: Unger 1990) and induces heterochromia (depigmentation) of the iris in pigmented rabbits (Ehinger et al. 1969; Ruskell 1982).

Because the iris is richly supplied with sympathetic nerve fibres (Ehinger et al. 1969;
unilateral surgical sympathectomy was performed in order to determine whether there is any involvement of sympathetic nerves in the regulation of ocular hyaluronan production in both normal and traumatized rabbit irises.

**Material and Methods**

Pigmented adult rabbits of mixed strain were used in this study. All animals were treated according to ARVO regulations for the use of animals in research. The experiments were monitored by the Swedish Board for Animal Experimental Ethics. Animals were anaesthetized with a mixture of ketamine (50 mg/mL; Ketalar®; Parke Davis, Solna, Sweden) and xylazine (20 mg/mL; Rompun®; Bayer, Göteborg, Sweden). An initial intramuscular injection of 1 mL/kg of the mixture was followed by subsequent injections as required.

Ulnar sympathetic denervation was performed by removing the right superior cervical ganglion. A careful dissection of structures was carried out to ensure that the cervical sympathetic ganglia were clearly identified. The animals were killed at 1 day, 3 days and 2 weeks after surgery with an intravenous overdose of pentobarbital. Each group consisted of six or seven animals unless stated otherwise.

In order to investigate the influence of sympathetic denervation on hyaluronan response in an injured iris, we performed a peripheral iridectomy of the iris in six denervated eyes 2 weeks after the removal of the superior cervical ganglion. These animals were killed 2 days later. Eight normal rabbits, four of which had undergone peripheral iridectomy, served as controls.

All rabbits were operated on the right side (ganglionectomy, peripheral iridectomy), leaving the left eye available as a control eye. The eye trauma (peripheral iridectomy) was performed in the upper iris half (at 12 o’clock) with scissors after the 3 mm corneal incision had been made with a keratome. The wound was closed with a 10-0 nylon suture.

The aqueous samples were collected with a 30-gauge needle inserted through the limbal cornea with the eye still in its socket. The cornea was then removed and iris tissue was obtained with scissors. The iris samples from both the unoperated control animals and the rabbits that had undergone iridectomy were divided into upper and lower halves. The aqueous samples were frozen immediately and stored at −20°C. They were later analysed for hyaluronan and total proteins without pretreatment. The iris samples were weighted wet and were also stored at −20°C.

The iris samples were freeze-dried for 48 h and then treated with pronase, which facilitates the subsequent extraction of hyaluronan from the tissue (Molander 1994; Johnsson et al. 1998). Hyaluronan concentrations were measured using a radiometric assay based on specific hyaluronan binding protein (Pharmacia HA test 50; Pharmacia & Upjohn, Uppsala, Sweden) (Brandt et al. 1987). Total protein concentration was determined by a turbidimetric procedure using benzethonium chloride precipitation as described by Luxton et al. (1989) and modified for automated analysis using a Hitachi 917 multipurpose analysis machine.

The aqueous flare was measured with a photoelectric instrument (Bengtsson et al. 1975) and expressed in arbitrary units. There is known to be a strong correlation between protein concentration and flare density (Anjou & Krakau 1961).

All results are given as the average ± SEM (standard error of the mean). Student’s t-test was used for statistical analyses, and the difference between unpaired groups was considered significant when p < 0.05.

**Results**

**Iris hyaluronan**

Hyaluronan concentrations in the irides of normal, non-treated rabbits were found to be 40 ± 5.0 μg/g wet weight. There were no statistically significant differences between the upper and lower halves of the irides or the right and left eyes.

In the group of animals that underwent peripheral iridectomy 2 weeks after sympathetic denervation, iris hyaluronan increased again in the upper iris halves, reaching 253.5 ± 30.5 μg/g wet weight 3 days after surgery, as assayed in the upper iris halves. This increase is statistically significant in comparison with observations of the upper iris halves in the normal control animals (p = 0.0002). Hyaluronan concentrations had returned to normal by 2 weeks after denervation. The iris hyaluronan of the left eyes remained unchanged.

In the group of animals that underwent peripheral iridectomy 2 weeks after sympathetic denervation, iris hyaluronan increased again in the upper iris halves, reaching 253.5 ± 30.5 μg/g wet weight 3 days after ocular surgery (p = 0.0032 compared to normal controls). In the denervated eyes, iris hyaluronan reached 296.3 ± 36.2 μg/g wet weight 2 days after peripheral iridectomy (p = 0.0027 compared to normal controls). There was no statistically significant difference between the increased hyaluronan concentrations 2 weeks after superior cervical sympathectomy plus an additional 2 days after peripheral iridectomy.
concentrations after iris trauma in the de-
nervated and non-denervated eyes. There
were no changes in the contralateral left
eyes (Fig. 1.)

The significantly higher concentrations of
hyaluronan after iris trauma were found
exclusively in the upper (iridectomized)
halves of both denervated and non-denerv-
ated eyes. Hyaluronan concentrations in
the lower halves did not show any signifi-
cant differences from those of the normal
control eyes in any of the different groups
(tauromatized denervated eyes: 44.17 ±
10.6 μg/mL; traumatized non-denervated
eyes: 67.8 ± 13.5 μg/mL; contralateral left
eyes in both denervated and non-denerv-
ated animals: 58.17 ± 12.5 μg/mL and 35
± 4.7 μg/mL, respectively).

Aqueous hyaluronan and aqueous flare
proteins

Hyaluronan concentrations in the aque-
ous increased slightly in right eyes 1 day
after denervation, from an average nor-
mal concentration of 0.79 ± 0.06 μg/mL
to 1.15 ± 0.1 μg/mL (p = 0.0491). No sig-
nificant difference from normal values
was found in hyaluronan levels 3 days and
2 weeks after removal of the superior cer-
cival ganglion.

In the denervated eyes, hyaluronan
concentrations increased to 2.31 ± 0.11
μg/mL 2 days after peripheral iridectomy
(p < 0.0001 compared to normal eyes).
This was lower (p = 0.0272) than the level
found in non-denervated eyes 2 days after
the same type of iris trauma (2.72 ± 0.1
μg/mL; p < 0.0001 compared to normal
eyes). The contralateral left eyes did not
show any significant changes in aqueous
hyaluronan (Fig. 2.).

Aqueous protein concentrations in-
creased rapidly to a maximum of 16.6 ±
2.5 g/L 1 day after denervation. This in-
crease is statistically significant (p =
0.0027) when compared to the aqueous
protein concentrations found in the nor-
mal control eyes (0.43 ± 0.08 g/L). These
concentrations subsequently decreased
swiftly, reaching normal levels by day 3.
Two days after peripheral iridectomy was
carried out in denervated eyes, protein
concentrations had increased again to
2.53 ± 0.5 g/L (p = 0.0208 compared to
normal eyes). This is not statistically dif-
ferent from the levels found following the
same trauma in non-denervated eyes
(2.09 ± 0.1 g/L). Aqueous protein levels in
contralateral left eyes remained un-
changed (Fig. 3.).

Changes in the aqueous flare were
similar to those noted in levels of aque-
ous proteins, with a normal value of
1.75 ± 0.5 arbitrary units and a high
peak value of 24.5 ± 4.5 units 1 day after
denervation (p = 0.0039 compared to
normal eyes). Two days after iridectomy,
the aqueous flare had again increased in
both denervated eyes (11.3 ± 1.1 units, p
< 0.0001) and non-denervated eyes (8.5
± 1.0 units, p = 0.0007). However, there
was no statistically significant difference
between the increases in denervated and
non-denervated eyes. Contralateral left
eyes did not show any significant
changes (Fig. 4.).

Two days after the iris trauma (periph-
eral iridectomy), the relatively low levels
of protein concentration in the aqueous
humor and low levels of aqueous flare re-
sponse in both denervated and non-de-
nervated eyes were, as expected, already
decreasing. The blood-aqueous barrier
recovers around 75 min after trauma in
both normal and denervated eyes (Kroot-
ila et al. 1987).

Fig. 2. The course of aqueous hyaluronan concentrations at different times before and after su-
perior cervical sympathectomy (column pairs A to D). Column pair E shows concentrations 2
weeks after superior cervical sympathectomy plus an additional 2 days after peripheral iridectomy.
Column pair F shows concentrations 2 days after iridectomy in non-denervated animals. Asterisks
indicate statistically significant higher levels than seen in non-denervated eyes (*: p < 0.05, **: p
< 0.02, ***: p < 0.01).

Fig. 3. The course of aqueous protein concentrations at different times before and after super-
ior cervical sympathectomy (column pairs A to D). Column pair E shows concentrations 2
weeks after superior cervical sympathectomy plus an additional 2 days after peripheral iridectomy.
Column pair F shows concentrations 2 days after iridectomy in non-denervated animals. Asterisks
indicate statistically significant higher levels than seen in non-denervated eyes (*: p < 0.05, **: p
< 0.02, ***: p < 0.01).
Discussion

Sympathetic denervation of the eye induces an acute response similar to that induced by trauma, and later enhances the response to ocular trauma (Koralewska-Makar et al. 1998, 2001). In the present study, concentrations of hyaluronan in the iris 2 days after ocular surgery were not significantly different in denervated and non-denervated eyes. The current experiments therefore do not support any assumption that sympathetic response to trauma by increasing its hyaluronan content.

Two weeks after denervation, hyaluronan concentrations in the iris had decreased after denervation degeneration of iris neurons, which may release neuronal hyaluronan as well as several different substances including prostaglandins known to stimulate de novo synthesis of hyaluronan in connective tissue. The sympathetic denervation does not appear to influence the iris hyaluronan response to trauma (iridectomy) or the distribution of hyaluronan in the iris tissue after trauma. From a clinical point of view, these results raise no particular concerns about the status of the sympathetic innervation of the eye in anterior segment surgery.

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