Superior Cervical Ganglionectomy in Monkeys: Surgical Technique

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Seven cynomolgus monkeys underwent a histologically confirmed left superior cervical ganglionectomy (SCGx). Unilateral ocular sympathetic denervation persisting for at least 2 yr was confirmed by ipsilateral ptosis, miosis, supersensitivity of pupillary dilation to topical phenylephrine, and profound pupillary hyporesponsiveness to topical hydrioxymetaphetamine. Intraocular pressure 8-9 and 23-27 months postoperatively were identical in the denervated and contralateral control eyes. This model should facilitate studies of aqueous humor physiology and pharmacology. Invest Ophthalmol Vis Sci 33:247-251, 1992

Decreased intraocular pressure (IOP) following disease-associated ocular sympathectomy was first reported by Horner. However, a modern study of patients with Horner's syndrome revealed ipsilateral IOP only ~1 mmHg lower than contralateral.1 Previous efforts to understand the role of sympathetic innervation in anterior segment physiology employed chemical approaches to create denervation or employed rabbits as the experimental animal.2-5 However, to delineate mechanisms most relevant to man, the studies should be conducted in a primate and the potentially confounding effects of ocular manipulation and administration of neurotoxic or other pharmacologic agents should be avoided.6 We describe here a survival technique for surgical superior cervical ganglionectomy (SCGx) in the living cynomolgus monkey.

Materials and Methods. Seven young adult female cynomolgus monkeys (Macaca fascicularis, 2.0-2.5 kg) were studied according to the ARVO Resolution on the Use of Animals in Research. Anesthesia for biomicroscopy, tonometry, and pupillometry was intramuscular (IM) ketamine 10 mg/kg, supplemented by 5 mg/kg every 30-60 min as needed. Anesthesia for SCGx was IM ketamine 10 mg/kg and IM pentobarbital Na 35 mg/kg.

Under full surgical depth anesthesia, the animal is placed in a right lateral decubitus position with neck extended. The left neck region is shaved and washed with an antimicrobial skin cleanser, after which the surgical site is painted with povidone-iodine solution and draped. The operative procedure is begun with an incision dorsal and parallel to the ascending ramus of the mandible, from just below the base of the ear lobe to the angle of the mandible. The superficial fascia is dissected and the ventral border of the sternocleidomastoid muscle is exposed. The external jugular vein, if in the field, is ligated and cut. The carotid triangle, bordered by the sternocleidomastoid, digastric, and omohyoid muscles, is entered and the tissues are dissected, with care taken not to cut the hypoglossal and cervical cutaneous nerves. The bundle containing the vagus nerve, internal jugular vein, and the carotid artery is located within the carotid triangle. The sympathetic trunk and superior cervical ganglion lie close to the vagus nerve at or above the level of the carotid bifurcation immediately ventral to the base of the skull. The ganglion and its connections are dissected free and cut, and the entire ganglion is excised, with care taken to include a small length of the sympathetic trunk cranial and caudal to the ganglion.

The subcutaneous fascia and skin are closed with running 4-0 polyglactin and polypropylene sutures, respectively. The operative procedure requires approximately 1.0 hr with the help of one assistant. Post-operatively the animal is isolated in a recovery cage and warmed with a heat lamp until fully conscious, and given food and water ad libitum. Systemic antibiotics (penicillin G benzathine and penicillin G procaine 30,000 IU/kg IM every other day for a total of three doses) and analgesic medication (butorphanol tartrate 0.2-0.3 mg/kg subcutaneously twice daily if indicated) are administered.

All seven animals tolerated the surgical procedure well. They were awake and alert in their cages on the first post-operative day. Skin sutures were removed on the 10th day. There were no wound infections.
One animal expired approximately 3 mo after the surgical procedure, on the day following an experiment. It had never fully awakened and death was believed to be anesthesia related. An autopsy was not performed. To date, we have been following the other six animals for 24–28 mo.

In all cases, the excised tissue was processed for light microscopy and embedded in paraffin. The tissue was sectioned in 5.0 μm-thick slices and stained with hematoxylin and eosin.

IOP was determined with a minified Goldmann's applanation tonometer. Pupil diameter was measured to the nearest 0.1 mm with a vernier caliper under normal room light (~350 lux). Differences between eyes and/or over time were evaluated for statistical significance by the two-tailed paired t-test or analysis of variance for repeated measurements (ANOVA-RM).

Phenylephrine HCl (PE; Sigma Chemical Co., St. Louis, MO) was prepared as a 0.1% solution in 0.9%

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Fig. 1. Excised SCGx specimen. (A) Cross-section of ganglion, showing intact capsule. (B) Typical sympathetic neurons within the ganglion. Hematoxylin and eosin (original magnifications: A ×60, B ×485).
NaCl. Six 5-μl drops separated by 5 min were applied to the central cornea of both eyes of the supine animal, delivering 30 μg of drug over 25 min. Pupil diameter in the prone animal was measured every 5 min for 1 hr, beginning just before the first drop. Commercial 1% ophthalmic hydroxyamphetamine HBr solution (HA; Paredrine; Smith Kline & French Laboratories, Philadelphia, PA) was applied to the central cornea of both eyes of the supine animal as seven 10 μl drops separated by 5 min, delivering 700 μg of drug over 30 min. Pupil diameter was measured as in the PE experiments. The quantitative HA and PE experiments were performed 24-28 mo, respectively, after SCGx. A qualitative HA experiment was performed at 1 mo, and qualitative PE experiments were performed monthly. A truncated (5 hr) diurnal IOP curve was determined under ketamine anesthesia 8-9 mo after SCGx. There was no special rationale for the experimental sequence. Other noninvasive experiments to be described elsewhere were performed at various times during these 28 mo. At least 2-3 weeks elapsed between successive experiments.

Results. In all seven surgical specimens, sympathetic neurons surrounded by an intact capsule were present (Fig. 1). While fully conscious, all seven animals exhibited relative miosis (~0.5 mm) and blepharoptosis (~1 mm) (Fig. 2A) on the SCGx side. Under ketamine anesthesia, the sympathectomized left pupil was a statistically significant ~0.2-0.5 mm smaller than the right at rest (Fig. 2B; Fig. 3 at time = 0; P < 0.01, two-tailed paired t-test). Over a 1 hr time period during and following topical HA (Figs. 2C, 3A), the SCGx pupil exhibited a small but statistically
cally significant 0.5 mm dilation \( (P < 0.009 \text{ by ANOVA-RM}) \), while the control pupil dilated \( \sim 2.0 \text{ mm} \) \( (P < 0.0001) \). The difference in responsiveness over the hour between the SCGx and the control eyes was highly significant \( (P < 0.001 \text{ by ANOVA-RM}) \). Over a 1 hr time period during and following topical PE (Figs. 2D, 3B), the control pupil dilated a small but statistically significant 0.5 mm \( (P < 0.01 \text{ by ANOVA-RM}) \), while the SCGx pupil dilated \( \sim 2.3 \) mm \( (P < 0.002) \). The difference in responsiveness over time between the eyes was highly significant \( (P < 0.007 \text{ by ANOVA-RM}) \). The resting miosis and blepharoptosis and the pupillary supersensitivity to PE were present within the first week following SCGx and persisted at monthly evaluation for the entire 28 mo observation period. The quantitative HA experiments were performed only more than 2 yr after SCGx, but qualitative experiments within the first two mo yielded similar findings. Under ketamine anesthesia, there was no difference between SCGx and control eyes in resting IOP over a 5 hr observation period 8-9 an 23-27 mo post-operatively (Fig. 4).

Discussion. Despite the vast literature dealing with the role played by adrenergic mechanisms in aqueous humor dynamics and other aspects of anterior segment physiology, much remains unknown. For instance, while \( \beta_2 \)-adrenergic receptors are present on trabecular, \( ^7 \) ciliary muscle, \( ^8 \) and nonpigmented ciliary epithelial cells, \( ^9 \) it is not clear whether any or all of these receptors are associated with a sympathetic neuroeffector junction. One investigative approach has been to observe the behavior of the system in the presence and absence of its sympathetic innervation. This has been achieved in rabbits using surgical sympathectomy \( ^2-^5 \) and in primates with pharmacologic sympathectomy \( ^4 \) or neurotransmitter depletion. \( ^6 \) However, given the significant anatomic and physiologic differences between the rabbit and human aqueous formation and drainage apparatus and the possible confounding effects of repeated pharmacologic assault, these models are not ideal. It seemed preferable to produce permanent sympathetic denervation in a primate with a one-time surgical procedure remote from the eye and requiring no pharmacologic adjuncts. With adequate knowledge of neck anatomy, SCGx in the cynomolgus monkey is a short, simple, straightforward, and well-tolerated surgical procedure. We are aware of only one prior mention of SCGx in the cynomolgus monkey is a short, simple, straightforward, and well-tolerated surgical procedure. We are aware of only one prior mention of SCGx in the monkey, involving a single rhesus being studied for visual deprivation myopia. Neither the surgical procedure nor any physiologic findings were described. \( ^{10} \)

Following sympathetic lesions, nonadrenergic nerves can acquire sympathetic markers and there also can be sympathetic input from other sources. \( ^{11,12} \)

Fig. 4. Intraocular pressure (IOP; mean ± SEM mm Hg) under ketamine anesthesia in normal (solid circles) and sympathetically denervated (open squares) eyes of six cynomolgus monkeys 8–9 months (A) and 23–27 months (B) following unilateral SCGx. Time 0 is 0900 hours, ~20 min after intramuscular ketamine, 10 mg/kg. No statistically significant difference between denervated and control eyes by the two-tailed paired t-test.

Therefore, although ganglionectomy was complete as evidenced by histology of the excised tissue in all of our cases, anatomic proof of complete ocular sympathectomy awaits morphologic and histochemical confirmation after in vivo studies have been completed. However, the persistent blepharoptosis and miosis, the pupillary supersensitivity to PE, and the poor pupillary response to HA indicate that functional denervation of the eye was essentially complete. \( ^{13} \) The minimal time-dependent mydriasis in the HA-treated SCGx eye (Fig. 3A) and the untreated normally innervated eye (Fig. 3B) might have represented marginally diminishing parasympathetic tone or increasing circulating catecholamine levels during the experiments, or, in the SCGx eye, weak residual sympathetic inner-
vation via standard or unknown alternative pathways, among many possibilities.

IOP under ketamine anesthesia fluctuated during a 5 hr observation period, but the values in the denervated and contralateral control eyes never differed significantly. Ketamine elevates sympathetic tone by blocking the presynaptic neuronal membrane pump, which mediates reuptake of neurotransmitter into the adrenergic nerve endings. Although the innervated control eye would be under enhanced sympathetic tone while the SCGx eye would remain atonal, the denervated SCGx eye would be supersensitive to circulating catecholamines. Furthermore, IOP is a function of many parameters, including trabecular outflow facility, uveoscleral outflow, episcleral venous pressure, and aqueous humor formation. The aggregate effect of sympathetic denervation on these parameters in the presence of ketamine could be such that IOP is changed very little. The unilaterally sympathectomized cynomolgus monkey should help delineate the role of the ocular sympathetic innervation in maintaining normal aqueous humor physiology and its response to pharmacologic agents.

Key words: intraocular pressure, monkey eye, pupil, superior cervical ganglionectomy, sympathetic denervation

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