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Research article

An experimental study of the neurophysical mechanisms of photophobia induced by subarachnoid hemorrhage

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HIGHLIGHTS

- In our previous studies, we have reported that the parasympathetic preganglionic denervation of the CG can occur in patients with SAH or meningitis as the result of an arterial rupture in the subarachnoid space.
- The purpose of this study was to investigate the potential relationship between SAH-induced neuronal degeneration in the CG, pupil diameters and photophobia scores in rabbits.
- In our previous studies in SAH animal models, we studied the relationship between the neuronal density in the autonomic ganglia of cranial nerves and vasospasm in the cerebral arteries.
- This study could contribute to a better understanding of the pathways involved in SAH-induced photophobia.

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ABSTRACT

Background: Photophobia is defined as a painful psychosomatic discomfort triggered by intense light flow through the pupils to the brain, but the exact mechanism through which photophobia is induced by subarachnoid hemorrhage (SAH) is not well understood. In this study, we aimed to investigate whether there was any relationship between the mydriasis induced by the degeneration of the ciliary ganglion (CG) and photophobia in instances of SAH.
Materials and methods: Five of a total of 25 rabbits were used as the intact control group; five were used in the sham-operated control group; and the remaining 15 were used as the SAH group, which was created by injecting autologous blood into their cisterna magna. All animals were examined daily for 20 days to evaluate their level of photophobia, after which their brains, CGs and superior cervical ganglia (SCGs) were extracted bilaterally. The densities of normal and degenerated neurons in these ganglia were examined by stereological methods.
Results: In SAH animals with a high photophobia score, the mean pupil diameter and density of degenerated neurons density in the CG were greater than in cases with a low photophobia score ($p < 0.05$). Further analysis revealed that the increase in the density of degenerated neurons in the CG following SAH resulted in the paralysis of the parasympathetic pathway of the pupillary muscles and mydriasis, which facilitates the excessive transfer of light to the brain and photophobia.
Conclusion: Our findings indicate that SAH results in a high density of degenerated neurons in the CG, which induces mydriasis and is an important factor in the onset of photophobia. This phenomenon is likely due to more light energy being transferred through mydriatic pupils to the brain, resulting in vasospasm of the supplying arteries.

1. Introduction

The pupils play a critical role in transmitting light between the outside world to the brain. The quality of images is optimized by the

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pupillary light reflex, in which the pupil constricts when the light intensity increases and pupil dilates when light intensity decreases. The photoreceptor cells of the retina include the rods, which are specialized for black and white vision in low light, and the cones, which are specialized for color vision in bright light [1]. The retinal ganglion cells projecting to the olivary pretectal nucleus also include a major projection to the Edinger-Westphal (EW) nucleus, which exerts parasympathetic action on the iris musculature via the ciliary ganglion (CG) [2]. The majority of the parasympathetic preganglionic motor neurons originating from the EW nucleus reach the CG via the oculomotor nerve (CN3), while a few reach the CG via the trigeminal nerve [2]. The terminal motor neurons of the pupils are located in the CG [3]. Anatomically, the CG is a small ganglion that is less than 2 mm long and resides within fat-filled connective tissue in the posterior orbit, just anterior to the superior orbital fissure. In humans, the CG contains an average of 3000 neurons and has a diameter of 20–35 μm., and the neurons in the CG primarily mediate pupilloconstriction and accommodation via the ciliary muscles [4].

Photophobia is a major symptom following subarachnoid hemorrhage (SAH) and is observed in many ophthalmic and neurologic disorders in response to intense light. Regardless of the cause, specific activation patterns in the trigeminal system can be observed in the trigeminal ganglion, trigeminal nucleus caudalis, ventroposteromedial thalamus, and anterior cingulate cortex during photophobia [5]. In 1996, Chronicle and Mulleners [6] speculated that the afferents from retinal ganglion cells that innervate the posterior thalamus and visual cortex along with sympathetic system hyperactivity might be responsible for photophobia. Clinically, it is widely known that light can initiate pain sensations in the orbit and head, whereas bright lights can also lead to trigeminal stimulation that produces oculocephalic discomfort that varies from mild to intolerable pain [7]. Recently, it has been reported that bright light significantly increases both the frequency and amplitude of the blinking reflex in subjects with photophobia [8].

In our previous studies, we have reported that the parasympathetic preganglionic denervation of the CG can occur in patients with SAH or meningitis as the result of an arterial rupture in the subarachnoid space. This denervation results in the degeneration of CG neurons followed by a dilated pupil [9,10]. Based on our current knowledge, we hypothesized that this loss of parasympathetic innervation in patients with SAH would lead to a relative sympathetic hyperactivity and thus an increased influx of light to the eye due to the resulting pupil dilation.

2. Material and methods–

This study was conducted on a total of 25 New Zealand white rabbits. The animal protocols were approved by Atatürk University Ethics Committee, and the care of the animals and the experiments were conducted according to the guidelines set forth by the same ethics committee.

2.1. Experimental protocol

Pupil diameters were measured in all animals in light and dark environments with an ocular coherence tomography device (Pentacam 70700: Oculus, Wetzlar, Germany), and fundoscopic examinations were performed for three times a day for two days prior to inducing SAH. The light sensitivity of the animals with dilated pupils was evaluated in a single-blind fashion by one of the researchers (M.D.A.) using a modified photophobia scoring system with grades from 0 to 3 (0 = normal, 1 = mild, 2 = moderate, 3 = severe) [11], and the mean values were used in the statistical analysis.

Five animals were used as the intact control group for the anatomical and histopathological examinations of the CGs, the SCGs, the CN3s, the posterior communicating arteries (PCoA) and the brainstem. All animals were anesthetized by isoflurane administered through a face mask, followed by a subcutaneous injection of 0.2 mL/kg of the anesthetic combination (ketamine HCl, 150 mg/1.5 mL; xylazine HCl, 30 mg/1.5 mL; and distilled water, 1 mL) before surgery. During the procedure, a dose of 0.1 mL/kg of the anesthetic combination was used when required; balanced, injectable anesthetics were used to reduce pain and mortality. Autologous blood (1 mL) was taken from the auricular vein and injected over the course of 1 min into the cisterna magna of animals in the SAH group using a 22-gauge needle. In the sham-operated control group, 1 mL of physiological serum was injected into the cisterna magna. The animals in the control group were not subjected to any injections. The animals were followed for 20 days without any medical treatment while their photophobia scores were recorded daily and then the animals were sacrificed. The brains, eyes and superior cervical ganglions (SCGs) of all animals were extracted and stored in 10% formalin solutions for histopathological examination.

2.2. Pupillary light reflex measurements

The measurement of pupil diameter, called pupillometry, was performed on unanesthetized rabbits using an ocular coherence tomography device as described below in detail [Fig. 1]. The animals were adapted to the experimental protocol by daily testing for one week to reduce stress-related pupil responses. The recordings were conducted during the middle of the light period, Zeitgeber Time (ZT) 6-6.5. Following a period of dark-adaptation for at least 1 h, rabbits were held carefully by the loose skin at the back of their neck to minimize blinking activity, which could interfere with the recordings; to immobilize the animal while ensuring unimpaired respiration; and to ensure a feeling of safety and facilitate a relaxed state. The rabbits were then positioned with one eye opposed to the outlet of an integrating sphere. The sphere was created from a 15-cm diameter white plastic ball, and to prevent light from escaping and thus interfering with the night goggles, the ball was painted black on the outside. The eye was recorded with an infrared sensitive camera (model XLVM, DAGE-MTI, Michigan City, IN, USA) fitted with a zoom lens (18–108-mm F2.5, Olympus, Tokyo, Japan) coupled to the opposite side of the sphere. The sphere was constantly illuminated by infrared light from diodes mounted in the bottom of the sphere. The camera was connected to a computer and operated by Streampix software (Norpix, Montreal, Canada). Light stimuli were provided by a 100-W halogen lamp and transmitted along a quartz fiber optic coupled to a circle surrounding the camera outlet, which illuminated the entire eye (Volpi AG, Schlieren, Switzerland). The intensity of the light was regulated with a set of glass neutral density filters (Chroma, Bellows Falls, VT, USA), and irradiance measures ($\mu W/cm^2$) were adjusted with a calibrated VEGA laser power meter (Ophir, Jerusalem, Israel). The eye was digitally captured at a frequency of 10 images per second for 2 min, and the pupil diameter was measured using ImageJ software (vers. 1.42q, NIH, USA) and converted to area. To correct for individual variation, data were normalized to the dark-adapted pupil size. Our preliminary experiments revealed that the non-invasive measurement of pupil diameter using ocular coherence tomography can reveal the constriction or dilation of the pupil, called miosis and mydriasis, respectively, in rabbits [Fig. 2].

2.3. Anatomical examination

Morphological examinations of the brains showed that all PCoAs were localized to the superomedial sides of the sulcus of the CN3 and extended from the fusion point of the internal carotid arter-